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01 1. Patogenesi microbica e sistemi di virulenza

16 - Targeting Parvovirus B19: antiviral strategies reviewed

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Targeting Parvovirus B19: antiviral strategies reviewed

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Introduction

Parvovirus B19 (B19V), a human pathogenic ssDNA virus in the Parvoviridae family, is responsible for a wide range of clinical manifestations, including severe clinical manifestations in patients with underlying haematological disorders or with immune system deficits. Currently, there are no recognized antiviral drugs for B19V treatment therefore ongoing research is directed towards the identification of compounds inhibiting B19V replication. In our activity, different approaches to antiviral discovery have been followed until now: drug repositioning strategy, screening of known antiviral compounds for a possible activity against B19 and a serendipity approach in screening small chemical libraries of compounds with possible antiviral activity.

Methods

Two cellular systems can be used to support viral replication in vitro and study the antiviral activity of tested compounds: the cell line UT7/EpoS1, although permissiveness is restricted to only a subset of cells, and primary erythroid progenitor cells (EPCs) that more closely resemble the natural target cells within the bone marrow environment. The antiviral effects of tested compounds can be evaluated by qPCR-based assays, to measure the variation in the abundance of viral DNA or mRNAs following a course of infection. In situ hybridization assays for viral nucleic acids or immunological detection of viral proteins, can be used to measure variations in the frequency of productively infected cells.

Results

The drug repositioning approach identified Hydroxyurea, an antiproliferative drug for sickle-cell disease, as effective against B19V, while Methotrexate, a folic acid analogue and antagonist used as an anti-inflammatory drug, showed no antiviral activity. Nucleotide analogues and its lipid conjugate, Cidofovir and Brincidofovir, demonstrated the most effective antiviral activity against B19V. Platinum-conjugated nucleotides with anticancer activity were investigated but are limited by cytotoxicity to EPCs. Foscarnet's clinical use has been reported, but only a low in vitro efficacy has been confirmed. The serendipity approach, based on screening small chemical libraries of compounds with potential antiviral activity, led to the discovery of

promising coumarin derivatives. Lastly, Ivermectins/Avermectins were shown to block the nuclear import of viral NS1 protein, inhibiting replication.

Discussion and conclusions

The results obtained suggest that various drugs, known for their use also in other therapeutic areas, could potentially be repurposed aginst B19V. However, further studies are required to confirm the potential of these drugs and explore their clinical application.

34 - Unveiling the Phenotypic Diversity and Macrophage Interplay in Hypermucoviscous Klebsiella pneumoniae: A Key to Intracellular Survival?

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Unveiling the Phenotypic Diversity and Macrophage Interplay in Hypermucoviscous *Klebsiella pneumoniae*: A Key to Intracellular Survival?

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Introduction

Klebsiella pneumoniae (*KP*) is a Gram-negative, encapsulated pathogen causing a wide range of infections. Hypervirulent strains often exhibit a hypermucoviscous phenotype (HMVp), resulting from genetic and environmental factors. The capsule aids immune evasion by inhibiting complement activation and reducing phagocytosis. Although traditionally considered an extracellular pathogen, recent studies highlight the complex interactions between *KP* and macrophages. This study aims to investigate these interactions, focusing on the poorly understood HMVp.

Materials and Methods

From our *KP* collection, we identified K24-18, a bovine isolate with a positive string test, indicative of the HMVp, and abundant capsule production. After opsonization, macrophages were infected with K24-18 and a control strain, K23-75 (non-hypermucoviscous, NHMVp), at MOI 10. Extracellular *KP* were eliminated using Polymyxin B (32 µg/mL for 15 min, then 8 µg/mL

to maintain the culture). At 1, 6, 24, and 48 hours post-infection (p.i.), cells were lysed, diluted, and plated for CFU counting. Colonies from 24 h p.i. were subjected to serum susceptibility testing and short and long-read sequencing with hybrid genome assembly.

Results

In vitro infection assays showed that K24-18 adhered to and invaded macrophages, with bacterial load increasing from 6 to 48 h p.i. Two distinct phenotypes were observed at each time point: large hypermucoviscous (HMVp), string test-positive colonies, and small NHMVp, string test-negative colonies. At inoculation, HMVp predominated within the population; however, by 6 h p.i., the most stressful phase of infection, NHMVp had become the dominant phenotype. By 24 h p.i., both phenotypes were present and had reached a stable coexistence. Serum susceptibility testing revealed that HMVp were more resistant to complement-mediated killing than NHMVp. WGS confirmed both variants belong to the same clone of sequence type 969 and capsular type 124 and lack the *rmpA* gene.

Discussion and Conclusions

Our preliminary results show that the hypermucoviscous *KP* strain K24-18 can infect and replicate within macrophages and further highlight the co-occurrence of HMV and NHMV phenotypes within the same clonal population, which differ in complement sensitivity and may cooperate during infection. These outcomes suggest a bet-hedging strategy involving the two different phenotypic traits, aiding intracellular survival and persistence. Ongoing analyses aim to identify capsule-related genetic differences, while microscopy studies investigate *KP* localization and interaction within the macrophage. Further experiments will explore similar phenotypic dynamics across other *KP* strains from our collection.

35 - Characterization of Vancomycin-Resistant and Vancomycin-Variable Enterococci in a Hospital Setting

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Characterization of Vancomycin-Resistant and Vancomycin-Variable Enterococci in a Hospital Setting

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1. Introduction

Enterococci, particularly *Enterococcus faecalis* and *Enterococcus faecium*, represent a significant threat as hospital-acquired pathogens due to their ability to resist multiple antibiotics, including the critical treatment option vancomycin. This study aims to investigate the prevalence and genetic characteristics of Vancomycin-Resistant enterococci (VRE) and

Vancomycin-Variable enterococci (VVE) within a hospital environment. The findings highlight the urgent need for improved surveillance and targeted interventions.

2. Materials and Methods

The species were identified and their antimicrobial susceptibility was determined using the MALDI-TOF (Brucker Daltonics) and Phoenix system (Beckton Dickinson). The minimum inhibitory concrentrations (MICs) values were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. Whole genome sequencing (WGS) was performed using Illumina Miseq (Illumina, San Diego, California, Stati Uniti), while antimicrobial resistance and virulence genes were detected through *in silico* analysis using the ResFinder and VirulenceFinder tools. Core genome multilocus sequence typing (cgMLST) analysis was performed with Ridom SeqSphere+ software.

3. Results

The results indicated a high prevalence of VRE at 74.5% and VVE at 25.5% among the isolates. Phenotypic susceptibility testing showed resistance to several antibiotics, including ampicillin, teicoplanin, and high-level gentamicin. Genetic analysis revealed multiple resistance genes and virulence factors, demonstrating significant heterogeneity among the isolates.

The study highlighted the presence of the *van*HAX operon and Insertion Sequences (IS) associated with glycopeptide resistance. The cgMLST analysis identified seven distinct clusters, indicating various genetic backgrounds. The predominant Sequence Type (ST) was ST80, which is commonly found in several countries.

4. Discussion and Conclusions

This study emphasizes the significance of molecular screening for effective surveillance and infection control, particularly in specialized hospital environments. It also highlights the necessity of ongoing surveillance and targeted interventions to prevent the spread of resistant strains within healthcare settings.

This work was supported by grants from the Italian Ministry of Health through Ricerca Corrente, Linea 3.

37 - Third-Generation Cephalosporin-Resistant Uropathogenic Escherichia coli From Community- and Hospital-Acquired Infections Show High Level of Antibiotic Resistance and Specific Virulence Traits

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Third-Generation Cephalosporin-Resistant Uropathogenic *Escherichia coli* From Community- and Hospital-Acquired Infections Show High Level of Antibiotic Resistance and Specific Virulence Traits

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Introduction. *Escherichia coli* is a leading cause of both community-acquired and nosocomial infections. In particular, *E. coli* is responsible for 90% of all uncomplicated urinary tract infections (UTIs) and 65% of complicated UTIs. Among complicated UTIs, those caused by third-generation cephalosporin (3GC)–resistant *E. coli* strains, expressing extended-spectrum beta-lactamases (ESBLs), are on the rise. These strains show often a multidrug-resistant (MDR) phenotype, limiting the therapeutic options and the increasing incidence of MDR *E. coli* in Algeria is concerning. This study aims to compare the antibiotic resistance rates and profiles as well as the virulence traits between 3CG-resistant *E. coli* isolates, collected from Algerian

inpatients (IPs) and outpatients (OPs). **Material and Methods.** Our analyses include, phenotypic and genotypic resistance factor detection, strains classification by genotyping and phylogrouping, as well as genotypic and phenotypic virulence factor evaluation. **Results.** Among 42 *E. coli* isolates, 76.20% caused UTIs. ESBL producers (n = 35) carried all the *bla*CTX−M gene, while *bla*TEM was found in 69.04% of isolates. All isolates were MDR, and no significant differences in type and rate of antibiotic resistance were observed between IP- and OP-isolates. OP-isolates demonstrated greater virulence, exhibiting higher motility and biofilm production, compared to IP-isolates. Moreover, pathogenic Phylogroup B2 was prevalent among OP-isolates, while IP-isolates belonged predominantly to Phylogroup A. **Discussion and conclusions.** Our data suggest a uniform spreading of antibiotic-resistant genes within hospitals and communities. However, hospital environment selects for less virulent strains with increasing level of resistance; differently, communities host more virulent strains. This study highlights the urgent need to implement the surveillance of 3CG-resistant *E. coli* and to adopt the One Health approach to monitor the antimicrobial resistance (AMR) in the country.

41 - A Role for Intracellular Replication and Neutrophil Recruitment in pneumococcal meningitis

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A Role for Intracellular Replication and Neutrophil Recruitment in pneumococcal meningitis

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Introduction

Pneumococcal meningitis is the most severe form of meningitis with a high risk of sequelae, particularly in children. However, the precise nature of the pathogenesis of the disease remains unclear, especially on how the host interacts with the pathogen during the initial phases of CSF invasion. In this study, we aimed to understand the early dynamics of pneumococcal brain invasion and the inflammatory process.

Materials and Methods

We analysed human brain autopsy samples from two cases of pneumococcal meningitis using, anti-pneumococcal, anti-CD169 (perivascular macrophages) and anti-CD31 (endothelial) antibodies with fluorescence microscopy. To investigates the dynamics of pneumococcal brain invasion, CD1 mice were infected intravenously with *S. pneumoniae*. After 24 hours, mice were treated with cefazolin, an antibiotic that does not cross the blood-brain barrier but clears bacteremia. This enabled us to study the infection within the brain while preventing systemic infection. Fluorescence microscopy and three-dimensional reconstructions were used to analyze bacterial localization within the brain tissues. We then treated mice with anti-CD31 and anti-CD169 antibodies to assess the role of endothelial and macrophage interactions in disease progression. Additionally, Caspase-1 inhibition using VX-765 was used to investigate its effect on bacterial release and inflammation.

Results

In the human brain biopsy samples, *S. pneumoniae* was detected within perivascular macrophages. Three-dimensional reconstructions revealed intracellular bacterial clusters, some of which showed signs of macrophage lysis. In the brain of infected mice, we found that in the first 24 hours, bacteria were associated with endothelial cells. At later time points, bacteria were localized within endothelial cells but also perivascular macrophages. Over time, bacterial clusters moved to an extracellular location, coinciding with macrophage lysis. This suggests that intracellular replication is a step preceding bacterial release and inflammation. Treating mice with anti-CD31 and anti-CD169 antibodies reduced the bacterial burden in the brain, highlighting the critical role of endothelial and macrophage interactions. Additionally, Caspase-1 inhibition with VX-765 prevented the appearance of extracellular bacteria, suggesting that pyroptosis plays a key role in bacterial release.

Discussion and Conclusions

Our results demonstrate that perivascular macrophages are central to the early stages of *S. pneumoniae* infection in the brain. The intracellular replication of bacteria within macrophages, followed by bacterial release into the extracellular space, appears to trigger a strong inflammatory response, including neutrophil recruitment. Importantly, inhibiting Caspase-1 to block pyroptosis may prevent bacterial release and mitigate inflammation in pneumococcal meningitis. These findings provide important insights into the pathogenesis of the disease and offer new options for therapeutic intervention.

42 - Phenotypic evolution of virulence factors of two Pseudomonas aeruginosa coexisting clones during cystic fibrosis airways infection

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Introduction:
P. aeruginosa is frequently isolated from cystic fibrosis (CF) patients, where it establishes chronic infections leading to high morbidity and mortality. During its adaptation to the CF lung, *P. aeruginosa* undergoes changes allowing antibiotic resistance and the development of differentiated virulence profiles. Whole genome sequence of *P. aeruginosa* strains collected from CF patients allowed to identify two different clones, named ST274 and ST324, that stably coexist and correlate with poor prognosis. When ST274 coexists stably with clone ST3243, ST274 is exclusively mucoid and ST3243 exclusively non-mucoid. In this study, we characterized 58 strains from 6 patients belonging to high-risk epidemic clone ST274 and 119 clones from 14 patients belonging to hypermutant multi-resistant clone ST3243. Our attention has been focused on the phenotypic characterization of virulence factors under variable oxygen conditions.
Materials and Methods:
A phenotypic characterization of virulence factors of 177 strains was performed in terms of biofilm formation, motility capability and pigment production under variable oxygen conditions. Biofilm production and motility were analyzed in different oxygen condition: aerophilia, microaerophilia and anoxia, while pigment production was analyzed in aerophilia and microaerophilia conditions, because the presence of oxygen is required for the biosynthesis of pyocyanin, pyorubin and pyoverdine.
Results: Preliminary data mostly showed a low production of virulence factors for both ST274 and ST3243 clones. In detail, each bacterial strain was weak biofilm producer, with some exceptions when strains were grown in microaerophilic condition. Both STs showed a lower swarming and swimming ability compared to reference strain PA14. In particular, swimming motility increased over the time in each oxygen condition for both clones. Swarming motility increased over the time only for ST274 clone, while for ST3243 clone it remained unchanged. The production of molecules involved in oxidative stress response such as pyocyanin and pyoverdine was not evidenced for the most of tested strains. Conversely, ST274 seemed produce pyorubin, even if strain-dependent, while ST3243 clone did not produce this pigment.

Discussion and Conclusions: The colonization by P. *aeruginosa* in CF patients involves some complications especially at pulmonary level; the coexistance of ST3243 and ST274 clones is often associated at negative prognosis. The results also show high heterogeneity of phenotypic traits even for strains belonging to the same clone. Understanding of *P. aeruginosa* phenotypic evolution will allow tailoring therapeutic approaches to the patient based on the characteristics of the colonizing strain.

43 - JC Polyomavirus in neural oncogenesis: a possible trigger in the Wnt/β-catenin pathway

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JC Polyomavirus in neural oncogenesis: a possible trigger in the Wnt/β-catenin pathway

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Introduction: JC polyomavirus (JCPyV) is associated with progressive multifocal leukoencephalopathy (PML), but its plausible role in brain cancers is also disputed. One candidate to mediate cell transformation is the Large T antigen (LTAg), which has the capability to bind the Wnt pathway protein beta-catenin, thus deregulating the cell cycle. In the current study, we investigated the presence and molecular state of JCPyV in pediatric gliomas and the effects of virus-positivity on the Wnt pathway. Moreover, a cellular model *in vitro* was carried out to further elucidate the plausible contribution of JCPyV infection in brain tumor development. Materials and Methods: The prevalence of JCPyV DNA was examined in 93 gliomas by quantitative polymerase chain reaction (qPCR). Then, qualitative PCR was performed to analyze the Non-Coding Control Region (NCCR) and Viral Protein 1 (VP1). In parallel, after RNA extraction, *LTAg* and *VP1* genes were examined by reverse-transcription (RT) PCR. For *in vitro* studies, the human glioblastoma cell line, U87-MG, was infected with supernatants collected from previous transfection experiments and analyzed for JCPyV viral load by qPCR, for *LTAg* and *VP1* genes, and viral miRNAs by RT-qPCR. In addition, both in brain tissues and U87-MG cells, the levels of *beta-catenin*, *c-myc* and *cyclin D1* were examined by RT-qPCR. Results: JCPyV DNA was found in 27/93 (29%) gliomas with a mean viral load of $5.8x10^3$ copies/ml. The amplified NCCR revealed an archetype sequence, and VP1 reported a high degree of homology with the reference strain in all JCPyV-positive samples. The *LTAg* gene was reported in all JCPyV-positive tumors, whereas *VP1* and viral miRNAs were detected in 22/27 virus-positive biopsies. Over-expression of *beta-catenin*, *c-myc* and *cyclin D1* was observed in JCPyV-positive tissues compared to negative ones. Moreover, once the capability of U87-MG cells to support viral replication and transcription was assessed, the Wnt pathway

was examined, showing an upregulation of *β-catenin*, *c-myc* and *cyclin D1* in infected cells. Discussion and Conclusions: Our findings confirm the presence of JCPyV in brain tumors. Specifically, the early gene expression concomitant with no detection of late genes suggests that hampering of viral replication may be crucial for tumor development. Moreover, this study provides an *in vitro* model to investigate JCPyV replication in brain cancer, laying the basis for further investigation on JCPyV-mediated oncogenesis in the brain. The over-expression of *beta-catenin* and its target genes observed in both *in vivo* and *in vitro* experiments strongly suggests that JCPyV exploits the Wnt pathway to modulate cellular processes and potentially contribute to brain carcinogenesis.

44 - Antarctic marine bacteria as a source of molecules with anti-virulence activity against ESKAPE pathogens

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Introduction. Antarctic marine bacteria are a valuable source of molecules of biotechnological and pharmaceutical interest. Some of them, are shown to be capable of interfering with the main virulence factors of pathogens without compromising their viability. Therefore, the study of these microorganisms is particularly relevant to address the current health emergency related to antibiotic resistance. Specifically, Antarctic psychrophilic bacteria *Pseudomonas aeruginosa* TAE6080 and *Psychrobacter sp.* TAE2020 are shown to produce two different proteins, named cold-azurin and Omp38, respectively. These proteins were proved to have anti-adhesive activity against the opportunistic pathogen *Staphylococcus epidermidis*. In this study, the activity of these molecules was analyzed against two pathogens belonging to the ESKAPE group and responsible for serious nosocomial infections: *Klebsiella pneumoniae* and *Enterococcus* spp.
Materials and Methods. Cold-azurin protein was recombinantly produced using *Escherichia coli* as host and the protein was subsequently purified from the periplasmic content through adsorption chromatography. Recombinant Omp38 was recovered, purified and refolded from *E. coli* inclusion bodies. Cold-azurin and Omp38 proteins were analyzed to evaluate their antibiofilm activity on selected clinical strains of *K. pneumoniae* and *Enterococcus* spp. For each strain, the ability of each proteins to inhibit biofilm formation and to disrupt mature preformed biofilm was studied. Staining was performed with crystal violet and biofilm quantification was determined spectrophotometrically at 590 nm.
Results. Both proteins did not show antimicrobial activity. Both proteins showed an excellent activity in inhibiting biofilm formation on most of the clinical analyzed strains, with a percentage of inhibition lower than 50% compared to the untreated sample. In the specific case of cold-azurin, a promising activity was also identified against preformed biofilm, which is generally much more resistant and difficult to eradicate.

Discussion and Conclusions. The microorganisms that inhabit Antarctica live in extreme environmental conditions that challenge their survival. Various studies show how the molecules produced are particularly interesting for various biotechnological applications. The analysis conducted in this case revealed that cold-azurin and Omp38 are proteins with potential anti-virulence activity against most of *K. pneumoniae* and *Enterococcus* spp. analyzed strains. Therefore, it is interesting to further the study of these two proteins to evaluate their efficacy also against other pathogens belonging to the ESKAPE group.

52 - PROBIOTIC FORMULATION OF LACTOBACILLUS REUTERI SLG01, VITAMIN C AND ACEROLA SHOWS BACTERICIDAL AND ANTIBIOFILM ACTIVITY AGAINST STREPTOCOCCUS MUTANS DSM20523

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Probiotic formulation of *Lactobacillus reuteri* SLG01, vitamin C and acerola shows bactericidal and antibiofilm activity against *Streptococcus mutans* DSM20523

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Introduction: Dental caries is a prevalent chronic infectious condition that impacts on the healthcare costs globally. This multifactorial disease can be caused by alterations of the plaque microbiome, started by repeated exposure to excessive amounts of carbohydrates, promoting the growth of aciduric and acidogenic bacteria and leading to dysbiosis. The proliferation of the cariogenic *Streptococcus mutans* accelerates the caries process. Thus, treatments targeting *S. mutans,* including alternative strategies using probiotics, might be effective in preventing the development of dental caries.

Materials and Methods: The probiotic formulation of *Lactobacillus reuteri* SLG01, vitamin C, acerola (dried extract tit. 50% vitamin C) was tested against *S. mutans* DSM20523. Antimicrobial activity of *L. reuteri* SLG01 was assessed by deferred antagonism and spot-on-lawn assays. MIC and MBC of *L. reuteri* SLG01 cell-free supernatant (CFS), vitamin C and acerola were determined with the microdilution method. Time-kill assays were carried out to determine the bactericidal kinetics for each compound. The potential synergistic activity of these compounds was tested by checkerboard method, combining either CFS and vitamin C

or CFS and acerola, at scalar dilutions from 1 to 8X MIC values. Lastly, antibiofilm activity was tested at scalar dilutions for each component.

Results: The antimicrobial activity of *L. reuteri* SLG01 was tested by deferred antagonism and spot-on-lawn assay. The activity of the probiotic components against *S. mutans* showed MIC values of 25% for CFS, 9.3 mg/mL for vitamin C and 18.7 mg/mL for acerola. The MBC values were a dilution higher than MIC for CFS (50%), vitamin C (18.7 mg/mL) and acerola (37.5 mg/mL). Time-kill assay confirmed how all tested compounds had bactericidal activity equal to 99.9% (3log reduction from the initial inoculation) at 4, 6, 24h respectively for CFS, vitamin C and acerola. The Fractional Inhibitory Concentration Index (FICI) as a measure of synergy shows indifference for all combinations. FIC(I) ranged from 1.05 to 1.15 was also confirmed when the associations were tested at values 2, 4, 8XMIC. The biofilm production was impaired for all components, with a stronger activity by vitamin C and acerola, which showed a reduction also at concentrations lower than MIC values.

Discussion and Conclusions: Probiotic formulations containing *L. reuteri* SLG01, vitamin C and acerola extract exert a bactericidal effect, especially strong for CFS, and antibiofilm activity for vitamin C and acerola. Thus, the combination of these three components could be advantageous for their complementary effects, as a novel treatment against the development of dental caries by *S. mutans*.

53 - Small RNAs carried by Outer Membrane Vesicles produced by hlyF-positive Shiga toxin- producing Escherichia coli represent a complex mechanism to regulate the infection process

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Small RNAs carried by Outer Membrane Vesicles produced by *hlyF*-positive Shiga toxin-producing *Escherichia coli* represent a complex mechanism to regulate the infection process

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Introduction

Shiga toxin (Stx)–producing *Escherichia coli* (STEC) harboring virulence determinants typical of Extraintestinal pathogenic *E. coli* (ExPEC) are currently emerging in Europe as a cause of severe disease. Among the ExPEC features identified in STEC, the pR444_A-like plasmids carry *hlyF*, a gene associated with an augmented production of Outer Membrane Vesicles (OMV). OMVs

produced by *E. coli* strains have been shown to deliver toxins and small RNAs, but information on the latter genetic component is still scanty.

Materials and Methods

We investigated the small RNAs contained in the OMVs produced by two *hlyF*-positive STEC strains producing Stx2 belonging to O26:H11 and O80:H2 serotypes, isolated from a human case of Haemolytic Uraemic Syndrome (HUS) and from a milk sample, respectively. The OMVs were purified from overnight cultures using two sequential ultracentrifugation steps at 100.000 and 200.000 x *g* of 2h each. The OMVs collected were analyzed by Nanoparticle Tracking Analysis (NTA) and Electron Microscopy (EM) to define their morphology, size and concentration. The whole genome sequences of the two test strains and three additional *hlyF*-positive STEC strains were analyzed to identify sequences of small RNAs. Specific Real Time PCR assays were deployed to detect the small RNAs within OMVs.

Results

The EM and NTA characterization confirmed the presence of bilayered-particles and showed that the introduction of sequential centrifugations in OMVs' purification resulted in a cleaner preparation obtained at 200.000 x *g*, suitable for downstream small RNA investigations. We were able to identify the sequences of 27 putative small RNAs (shorter than 200 nucleotides) present in the genome of *hlyF*-positive STEC strains and absent in that of a non-pathogenic *E. coli* K12 strain, analyzed for comparison. For 12 small RNAs it was possible to develop specific Real Time PCR assays. Nine of them were identified and quantified in the OMVs produced by the two test strains. A regulatory role in bacterial DNA replication, integration and in stress-response was hypothesized for the identified small RNAs, some of which encompass all the domains of life. STnc_100 was the only small RNA identified in the OMVs produced by both strains. Interestingly, a copy of STnc_100 sequence is harboured downstream the *stx2* operon carried by Stx2-encoding phages in both the strains and has a complementarity region for *stxB* gene, suggesting a modulation in Stx production or release.

Discussion and Conclusions

Our results indicate that OMVs release may exert regulatory functions, putatively influencing the crosstalk with the host and the gut microbiota during the infection process. Furthermore, our findings suggest that the small RNA STnc_100 may exert a role in the regulation of Stx production putatively mediated by OMVs delivery.

55 - Oral Microbiota and Inflammatory Profile Alterations in Children and Young Adults with Type 1 Diabetes

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Oral Microbiota and Inflammatory Profile Alterations in Children and Young Adults with Type 1 Diabetes

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Introduction. Type 1 diabetes (T1D) is a chronic disorder characterized by the autoimmune destruction of pancreatic beta-cells with absolute insulin deficiency. T1D causes short- and long-term complications, including oral inflammatory diseases. However, the complex interplay between T1D, local immune response and oral microbiota remains poorly understood, with limited evidence in the current literature. The study aimed to understand the interaction between oral microbiome and inflammatory markers in T1D by comparing children and young adults with T1D to healthy control (HC) subjects. **Materials and Methods.** We analyzed saliva samples from 75 children and young adults with T1D and 79 HC. Oral microbiome analysis was performed using 16S rRNA gene targeted sequencing on the Illumina platform, while salivary cytokine levels were quantified using magnetic bead-based multiplex immunoassays (Bio-Plex-200 system | Bio-Rad Corp., Hercules, CA, USA). **Results.** Alpha diversity analysis showed a significant reduction in species richness (p=0.0008) and an increase in species evenness (p=0.0001) in T1D compared to HC. Furthermore, beta diversity confirmed different community structures between the two groups. At the genus level, T1D individuals showed a decreased abundance of *Fusobacterium* (p=0.0008) and an increased abundance of *Atopobium* (p=0.011) and *Prevotella* (p=0.022). In addition, cytokine profiles

demonstrated significantly lower levels of Interferon-gamma (IFN-γ) ($p<0.0001$) and higher levels of Interleukin-1beta (IL-1β) ($p=0.01$) in T1D compared to HC. IL-1β levels were also positively associated with the relative abundance of *Prevotella* ($p=0.001$). **Discussion and Conclusions.** The increased abundance of pro-inflammatory genera, such as *Prevotella*, often observed in individuals with metabolic disorders including diabetes, may contribute to local immune dysregulation in T1D, as evidenced by altered cytokine profiles. The observed correlation between IL-1β and *Prevotella* abundance also supports a potential link between microbial shifts and inflammatory responses. In conclusion, our findings offer deeper insight into the role of the oral microbiome in modulating local inflammation in T1D, suggesting the importance of early and regular dental care in T1D individuals. However, further research is necessary to elucidate the underlying mechanism and the bidirectional relationship between oral microbiota composition, inflammation and T1D.

65 - Human genome integration of anelloviruses: a potential trigger for oncohematological cancers

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Human genome integration of anelloviruses: a potential trigger for oncohematological cancers

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Introduction: Over the past four years, cases of Torque Teno Mini Virus (TTMV) integration into the human genome have been reported. These rare events occur in the Retinoic Acid Receptor Alpha (RARA) gene, forming the TTMV-RARA fusion gene, which has been implicated in a variant form of acute promyelocytic leukemia (APL). Typically, APL is characterized by the PML-RARA fusion gene. The molecular characterization of TTMV-RARA-positive APL remains poorly understood, raising important questions about its role in leukemogenesis and the interaction between anelloviruses and humans.

Materials and methods: Two Italian APL cases, the first identified worldwide, were reanalyzed to characterize the TTMV-RARA chimeric transcripts. Bioinformatic analyses were conducted to identify the fusion transcript between TTMV and RARA from RNA-seq data. An Illumina target DNA-seq of the region around the integration site in intron 2 was conducted. Then, an anello-

specific Alu-PCR, was performed to confirm *in-silico* observations. The Alu-PCR was designed within a UTR region that also possesses high homology with other Anelloviruses, thus extending the search for possible integrations not only to TTMV and not only in the RARA gene.

Results: RNA-seq confirmed TTMV-RARA fusion transcript with a portion of retained intron 2; also DNA-seq confirmed this. In one of the two patients, bioinformatic analysis shows the integration of the almost complete genome of a TTMV (2869 bp) while in the second only a part (1040 bp). These, combined with other TTMV-RARA fusion cases and over 1000 TTMV isolates, deposited in NCBI, revealed a cluster of "integrated" TTMV strains. Transcript analysis shows the absence of stop codons in the integrated sequence, suggesting that these may be expressed as a fusion protein; these proteins could provide insights into this leukaemia variant. Alu-PCR showed a high level of the integrated TTMV sequence at the onset of the disease, a low level after haematopoietic stem cell transplantation (HSCT) and a return to the baseline level 8 months after transplantation.

Discussion and Conclusions: These findings challenge the characterization of anelloviruses as orphan viruses. Fourteen cases of TTMV integration into the RARA gene have been reported, with varying integration points that do not suggest homologous recombination. Integration probably occurs through micro-homology between the UTR region of anelloviruses and the human genome. Understanding the structure of proteins expressed by these transcripts could provide insights into their role in this leukemia variant. Extending the search for integrations to Torque Teno Viruses (TTV), the most prevalent anelloviruses in the general population, would be worthwhile.

71 - The role of FosB in Staphylococcus epidermidis fosfomycin resistance

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The role of FosB in *Staphylococcus epidermidis* fosfomycin resistance

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Introduction and objective

Fosfomycin is an antimicrobial compound that binds the enzyme MurA and impairs the biosynthesis of the cell wall. FosB is a bacillithiol-transferase that confers fosfomycin resistance. In *Staphylococcus epidermidis fosB* can be found both in the core genome (*cg*-fosB) and in accessory-elements (*ag*-fosB), with a nucleotide identity of 69%. *cg-fosB* is universally present albeit often carrying stop-codon mutations or frame-shifts. Our aim is to have a better understanding of the role of FosB in fosfomycin resistance.

Material and methods

We collected, sequenced and analyzed 147 blood-culture isolates and 39 carriage isolates of *S. epidermidis*. Fosfomycin susceptibility was tested using MIC Test Strips. Cloning was performed by using the DNA cytosine methyltransferase negative *E. coli* DC10B strain followed by plasmid extraction and electroporation into *S. epidermidis* ATCC 12228

Results

cg-fosB was present amongst all the isolates, but only eight clinical isolates showed the presence of *ag-fosB*. Isolates were tested for fosfomycin susceptibility and *ag-fosB* carrying isolates showed MICs of 128mg/L, while the other isolates showed MICs of 0.25-16 mg/L. *cg-fosB* mutations did not have any impact on the susceptibility. To test the phenotype conferred by the *cg-fosB,* we provided a second copy of *cg-fosB* to *S. epidermidis* ATCC 12228 by cloning the gene into the pRMC2 tetracycline-inducible plasmid. The recombinant strain showed a four-fold increase of the MIC to fosfomycin, from 0.5 mg/L of the wild-type strain to 8 mg/L in the recombinant.

Discussion and Conclusions

Our data indicate that fosfomycin resistance in the clinical isolates we tested was conferred exclusively by the *ag-fosB* gene. In contrast the *cg-fosB* provided resistance only when expressed under an inducible-promoter. Despite being constitutively silenced, *cg-fosB* is maintained in the *S. epidermidis*, pointing to the gene's potential involvement in other key functions.

75 - Gut microbiome characterization among African and Italian individuals with diarrheal diseases

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Gut microbiome characterization among African and Italian individuals with diarrheal diseases

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Background: The gut microbiota represents the most intricate, diverse, and densely populated microbial ecosystem within the human body. While its vital role in health and disease is well established, studies focusing on the diversity and functionality of the gut microbiome in vulnerable groups, such as migrants and/or refugees, remain scarce. This study aims to explore the gut microbiota diversity among African migrants/refugees arriving in Italy, and to compare their microbial profiles with those of the autochthonous Italian population.
Material and methods: Between 2022 and 2024, individual, non-repetitive stool samples were collected from 79 African migrants/refugees and 56 Italian individuals experiencing diarrheal illness, admitted at Policlinico Riuniti, Foggia, Italy. Age, sex, and country of origin was gathered for all the participants. DNA was extracted from faecal samples using the MagCore® Nucleic Acid Extraction Kit followed by amplification and sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene amplification and sequencing. Bioinformatics analysis was conducted using QIIME2, DADA2, and the SILVA database, assessing the relative abundance (RA) of the identified OTUs at both the phylum and genus levels.
Results: On average, over 23,000 sequences (mean of total length > 5.7Mbp) and more than 200 bacterial genera were obtained per sample. Significant differences in both inter-individual and intra-individual diversity were observed between the two groups, with wider variability observed in key bacterial taxa like *Bacteroides* spp. (RA: 4.4%-83.3%), *Firmicutes* spp. (RA: 3.5%-84.2%), *Proteobacteria* spp. (RA: 0.2%-91.8%), and *Verrucomicrobia* spp. (RA: 0%-21.5%). A higher *Clostridioides difficile* prevalence was detected within *Firmicutes* group in over half of the African migrants/refugee samples, highlighting a possible link to diarrheal symptoms and severe colonic damage.
Discussion: These findings provide crucial insights into the gut microbiota of an often overlooked, high-risk population. Given their frequent exposure to malnutrition and food

shortage, these individuals exhibit unique microbiome profiles. The study also supports comparative research with populations in high-income countries, emphasizing the influence of demographic and environmental factors on gut microbiome diversity and health. More in depth analysis with a higher sample size will contribute to improve the knowledge in the microbiome profile.

113 - Development of a 3D in vitro epithelial model to investigate host-pathogen interactions during Candida albicans and Herpes Simplex Virus 2 mono- and co-infections in a simulated vaginal environment

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Development of a 3D *in vitro* epithelial model to investigate host-pathogen interactions during *Candida albicans* and Herpes Simplex Virus 2 mono- and co-infections in a simulated vaginal environment

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Introduction. *Candida albicans* (Ca) and Herpes Simplex Virus 2 (HSV-2) are among the most prevalent pathogens affecting the human genital tract, frequently coexisting and potentially interacting in ways that influence infection dynamics and host responses. Increasing literature documents that the presence of a primary infectious agent can predispose the host to a secondary infection, suggesting a possible synergistic interplay between pathogens. We recently published the results of a study investigating Ca and HSV-2 mono- and co-infections of the A-431 epithelial cell line. These cells, derived from a human epidermoid skin carcinoma, form a monolayer after 24 hours of incubation or, after 5-day growth, a differentiated multilayer which closely mimics a reconstituted vaginal epithelium. Building on this foundation, the present study aims to develop a three-dimensional (3D) *in vitro* model of vaginal epithelium using A-431 cells cultured on inert scaffolds, enabling a detailed analysis of host-pathogen interactions during both single and dual infections, in the presence or absence of a synthetic vaginal fluid (SVF).

Materials and Methods. The A-431 epithelial cells were cultured on 3D inert scaffolds, incubated for 5 days in DMEM with 10% FBS, exposed or not to SVF, and infected with Ca and/or HSV-2. Epithelial viability was assessed by LDH release; fungal burden and viral load were also evaluated, at different time points and conditions, by CFU assay and PCR-based DNA quantification, respectively. Histological and immunohistochemical (IHC) staining with hematoxylin and eosin (H&E) and specific anti-cytokeratin 5/6 (CK 5/6) antibodies were performed on slides from the scaffolds.
Results. The A-431 line exhibited the ability to adhere to inert scaffolds and to grow as a multilayered epithelium, after 5 days of incubation, as shown by H&E staining. Specific IHC analysis revealed the highest levels of CK 5/6 expression in the presence of SVF, indicative of a high degree of epithelial cell differentiation. The addition of SVF enhanced the Ca survival and growth ability. Epithelial cell viability was significantly impaired by Ca infection, whereas HSV-2 had minimal or no effect; the dual infection resulted in slightly additive effects in terms of cell damage.
Discussion and Conclusions. The A-431 epithelial cells demonstrated the capacity to colonize and grow onto an inert 3D matrix, mimicking the structural and functional characteristics of human vaginal mucosa. Thus, the 3D inert scaffolds represent a useful tool to develop *in vitro* studies on polymicrobial infections, with the addition of SVF providing a prototype as similar as possible to the *in vivo* vaginal environment.

125 - Epidemiology of Anti-HCV Seroprevalence and RNA-HCV by Sex, Age and Hospital Unit in a Large Catchment Area in Campania(2016-2023): Implications for Hepatocellular Carcinoma Risk Management

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Epidemiology of Anti-HCV Seroprevalence and RNA-HCV by Sex, Age and Hospital Unit in a Large Catchment Area in Campania (2016-2023): Implications for Hepatocellular Carcinoma Risk Management

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Introduction: Hepatitis C virus (HCV) infection can progress to chronic liver inflammation, liver failure and hepatocellular carcinoma (HCC). HCC is an major complication of HCV-related cirrhosis with an average annual incidence of 1-4%. The development of HCC is mainly associated to two conditions: cirrhosis, characterized by ongoing necroinflammatory activity, and the failure of immune surveillance due to viral escape mechanisms. Due to these risks, monitoring the prevalence of anti-HCV antibodies and HCV RNA is critically important. This study, conducted at the San Giovanni di Dio and Ruggi d'Aragona Hospital in Salerno (2016–2023), aimed to assess HCV prevalence, stratifying patients by sex, age, and hospital department.

Materials and methods: The qualitative detection of HCV antibodies (anti-HCV) in human serum was performed using the VITROS Immunodiagnostic System. The assay measures signal intensity, which is directly proportional to the concentration of anti-HCV antibodies in the sample. Detection of HCV-RNA was conducted using polymerase chain reaction (PCR) to assess the presence or absence of viral RNA.
Results: A total of 116,478 samples were analyzed, of which 4,214 (3.6%), tested positive for anti-HCV antibodies, showing a significant decreasing trend over time. Although a greater number of samples were collected from females, only 1,797 (42.6%) were positive, compared to 2,417 positives (57.4%) in males. Age-stratified revealed that seroprevalence was highest in the 71-80-year age group for both sexes. The highest anti-HCV seroprevalence rates were observed in samples from Immunotransfusional (27.3%), the Department of Oncology (22.2%) and the General Medicine – Prisoner Section (22.1%). HCV-RNA testing was performed in 50% of patients who tested positive for anti-HCV antibodies, with an overall RNA positivity rate of 68.4%. The 71-80 years age group remained the most affected. Departmental stratification of HCV-RNA results revealed a 100% positivity rate in samples from the Obstetrics and Gynecology Clinic, Pediatric Clinic, Urology Clinic, Dialysis Unit, Stroke Unit, and Pulmonology Department. High RNA positivity rates were also recorded in the Infectious Diseases Department (86.7%) and the Emergency Medicine Department (81.6%).
Discussion and Conclusions: The study highlights a decreasing trend in HCV prevalence, reflecting advances in prevention and management. While the widespread use of direct-acting antiviral (DAA) therapy has significantly reduced the global burden of HCV infection, the risk of HCC persists, especially among patients with advanced liver damage. Maintaining targeted surveillance strategies, alongside a deeper understanding of the HCC development, is crucial to optimize prevention efforts and reduce residual risk.

141 - Postbiotics: a new therapeutic approach

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Postbiotics: a new therapeutic approach

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Introduction. Probiotics are live bacteria that, when administered in adequate quantities, exert beneficial effects on the host. Probiotic bacteria, through the digestion and fermentation of dietary fiber, produce a diverse array of bioactive compounds called "postbiotic metabolites", able to influence health and disease phenotypes. As postbiotics do not contain live microorganisms, the risks associated with their consumption are minimized. However, the metabolic activity of bacteria, and therefore their metabolites, changes rapidly according to habitat conditions: the same probiotic may be effective in one individual and ineffective or even harmful in another. Postbiotics represent a new frontier, not yet fully explored, to develop a different approach to health promotion. The aim of the present study was to investigate the bioactivity of postbiotics under different probiotics growth conditions. **Material and Methods.** Two probiotic strains, *Lactobacillus paracasei* and *Lactobacillus plantarum*, were grown under anaerobic and aerobic conditions and the cell-free supernatants (CFS) were collected at different phases of the growth curve (stationary and death phases). The CFS, as is and freeze-dried, were evaluated for: a) effect on the growth of collection bacterial (*Escherichia coli* MG1655 and *Staphylococcus aureus* ATCC 6538), and clinical isolates (Methicillin-Resistant *S. aureus* and *E. coli* ESBL), under planktonic and sessile conditions; b) effect on the growth of the probiotic strains themselves; c) toxicity towards the human intestinal cell line Caco-2; d)

effect on the adhesive/invasive ability of intracellular bacteria. **Results**. The bioactivity of the CFS was only detectable after their concentration by lyophilization. The antibacterial activity was highlighted only against potentially pathogenic strains, not against the probiotic ones. The bioactivity of CFS towards planktonic bacteria and preformed biofilm was different between the two probiotic strains, depending on the time of sampling, the strain tested and the pH of the culture. **Discussion and Conclusions.** The interaction with the host of probiotics occurs through metabolic products, and their action also differs depending on the habitat. The pH, temperature, oxygen tension and type of substrate influence bacterial metabolism, and consequently the metabolites produced. It is imperative to ascertain the optimal conditions for the proliferation of a probiotic strain, with the objective of yielding a mixture of bioactive compounds that exhibit a specific activity. Performing bacterial fermentation processes under controlled and standardized conditions represents a pivotal step in the production of CFS with specific bioactivity, enhancing the comprehension of their mechanism of action.

144 - Disruption of key virulence traits in Stenotrophomonas maltophilia by N-Nonyloxypentyl-L-Deoxynojirimycin (L-NPDNJ)

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Disruption of key virulence traits in *Stenotrophomonas maltophilia* by N-Nonyloxypentyl-L-Deoxynojirimycin (L-NPDNJ)

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Introduction: *Stenotrophomonas maltophilia* is an opportunistic Gram-negative pathogen that causes life-threatening infections in immunocompromised hosts and chronic lung infections in cystic fibrosis patients. Its clinical relevance has increased due to its intrinsic multidrug resistance and an arsenal of virulence factors. These include protease secretion, biofilm formation, the induction of host tissue damage and immune evasion, all of which contribute synergistically to its persistence and pathogenicity.

Material and Methods: A small library of synthetic iminosugars was screened against eight *S. maltophilia* strains, including the reference strain K279a. Antibacterial activity was assessed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration determination, while combination effects with aminoglycosides were evaluated using checkerboard assays. Biofilm formation and eradication assays were tested by crystal violet and tetrazolium salt reduction assay. Transcription of selected virulence-related genes was verified by qRT-PCR. Protease activity assays, adhesion assays on A549 epithelial cells, macrophage infection assay in RAW 264.7 cells, and an *in vivo* infection with *Galleria mellonella* larvae were also performed.

Results: The most active compound L-NPDNJ showed limited antibacterial activity but showed additive effects with aminoglycosides. It significantly disrupted preformed biofilms, with over 60% reduction in biomass and metabolic activity, although it did not inhibit initial biofilm formation. qRT-PCR analysis revealed a marked downregulation of genes encoding resistance determinants (smeC, aph(3')), biofilm regulators (rlmA, fsnR), and virulence factors including major proteases (sphB, stmPr1-3), hemolysins (smlt1704, smlt3638), and adhesion-related enzymes (estE, plcN1). These transcriptional changes led to reduced protease secretion, resulting in reduced epithelial damage by K279a supernatants, and impaired adhesion of *S. maltophilia* to A549 epithelial cells. We also observed that in the macrophage infection assay, treated bacteria exhibited increased phagocytosis and reduced intracellular survival compared to untreated cells. In addition, L-NPDNJ treatment significantly improved larval survival in the *in vivo G. mellonella* model, confirming its ability to attenuate virulence.
Discussion and Conclusions: Despite lacking direct bactericidal activity, L-NPDNJ effectively interferes with multiple virulence mechanisms in *S. maltophilia*, including protease secretion, biofilm maintenance, epithelial damage, and host colonization. These findings support its potential as a non-toxic antivirulence agent that could be used to complement antibiotic therapy and reduce pathogenicity without promoting antimicrobial resistance.

157 - Selective events in core genes and possible niche adaptation in Fusobacterium animalis

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Selective events in core genes and possible niche adaptation in *Fusobacterium animalis*

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Introduction

Fusobacterium animalis (*Fa*) was identified as the most enriched *Fusobacterium* species in colorectal cancer. Recently, a group of *Fa* core genes were found to be highly expressed intratumorally and to favor the intracellular survival. We hypothesized that, because they promote bacterial fitness in the intracellular niche, these genes might be targets of positive selection, a process that often underlies adaptation to variable environments. We thus performed an evolutionary analysis to identify selective events that occurred over different time frames, namely during the divergence of the *Fusobacterium* species and in the more recent separation of the *Fa* lineage from *F. paranimalis*.

Materials and Methods

We retrieved sequence information for the reference genomes of the *Fusobacteria* species most closely related to *Fa* (14 species) and we compared evolutionary models that allow or disallow codons to evolve under the presence of positive selection. We also applied a population genetics-phylogenetic approach to search for and detect beneficial changes that occurred in the *Fa* lineage.

Results

These genes have been targeted by intense purifying selection, possibly as the result of their essential functions. However, localized signatures of positive selection were also detectable. During the divergence of *Fusobacterium* specie, the major target of selection was represented by elongation factor-Tu, a finding that could be related to its moonlighting function in adhesion and biofilm development. Additional targets were rpoC and the septum-determining protein minD. We suggest that variations in the latter contribute to the observed differences in cell length and width between *F. watanabei* and *Fa*. We also searched for and detected beneficial changes that occurred in the *Fa* lineage, suggesting that such variants promote intracellular

growth or adaptation to the tumor microenvironment. The strongest target of selection was dnaK, which was shown to promote malignant transformation. Analysis of the selected sites in dnaK indicated that most of them determine the appearance of eukaryotic linear motifs (ELMs). Specifically, two ELMs are involved in SUMOylation and USP7-mediated deubiquitination. USP is a central modulator of the p53-MDM2 pathway and we suggest that SUMOylation facilitates the nuclear import of *Fa*.

Discussion and Conclusions

Our data show that the genes we analyzed, which are highly expressed in *Fa* positive tumors, are present in most *Fusobacterium* genomes and evolved under strong constraints. However, distinct *Fusobacterium* species are also adapted to different niches and the identification of specific amino acid changes that are expected to underlie phenotypic diversity is relevant to inform future analyses of *Fa* oncogenic potential.

158 - Long- and short-term evolution of Herpesvirus proteins: identification of functional and structural determinants

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Long- and short-term evolution of Herpesvirus proteins: identification of functional and structural determinants

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Introduction

Understanding the factors that affect and shape viral protein evolution is a central question in evolutionary virology, important for our ability to predict virus evolvability and the molecular pathways that can lead to emergence of new viruses. However, with the exception of a few studies, we lack comprehensive quantitative understanding of how different functional and structural characteristics are linked to viral protein evolutionary rates, and whether trends observed in cellular protein evolution differ from those in viral proteins.

Materials and Methods

Here, we take advantage of the phylogeny of the Orthoherpesviridae family – a large family of herpesviruses, that includes several biomedically important human herpesviruses that

originate in three distantly related genera. We focus on representative human viruses from each of the three genera (i.e.: HSV1, HCMV and KSHV), and employ various evolutionary models to study the long- and short-term evolution of their proteins, using viral isolates sampled from patients, closely-related primate-infecting herpesviruses from the same genus, and comparisons of orthologous core genes found in distant herpesviruses.

We link the evolutionary rates of the viral proteins and their residues to a large set of structural, cellular and functional properties, constituting the largest such analysis of a viral proteome to date.

Results

Core genes whose orthologs are found in distant genera, evolve at similar rates within genera, despite their evolutionary distance and their differences in viral replication and environments. This likely stems from constraints posed to maintain the structural fold that remains conserved across viruses, and is corroborated by the finding that fold complexity is a major determinant of evolutionary rates. Focusing on evolution of specific regions, we show that surface and disordered regions are enriched with positively selected residues. Within disordered regions, motifs that mediate binding to specific host proteins are highly conserved. Additionally, viral proteins predicted to form biomolecular condensates, that often involve disordered regions and their interactions with host proteins, tend to be conserved.

Discussion and Conclusions

These new findings add to the emerging view of the evolution of disordered regions, and how functional elements within them evolve and constrain protein evolution.

Our analyses reveal short- and long-term evolutionary constraints posed on herpesvirus proteins by their structural fold and on disordered regions, important for viral interactions. These constraints are relevant when considering potential pathways of viral evolvability and for developing new antiviral treatments.

165 - Study of new selected LasB inhibitors as an anti-virulence strategy against Pseudomonas aeruginosa

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Study of new selected LasB inhibitors as an anti-virulence strategy against *Pseudomonas aeruginosa*

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Introduction: Infections caused by multidrug-resistant *Pseudomonas aeruginosa* represent a serious threat to public health globally. In recent years, much research has focused on the development of alternative antibacterial strategies, including the use of antivirulence drugs. The present study aimed to evaluate *in vitro* the antivirulence properties of selected metalloprotease inhibitors. Such inhibitors were specifically designed to inhibit LasB elastase, which is a Zn-dependent metalloprotease secreted by *P. aeruginosa*, capable of hydrolysing a wide spectrum of host proteins and causing alterations in the immune response as well as tissue damage. **Materials and methods**: New inhibitors based on an N-benzyloxy-aminoacid-hydroxamate (BAH) scaffold were used. To evaluate the inhibitory effect of BAHs towards LasB, the elastolytic activity of secreted LasB was spectrophotometrically (OD: 495nm) measured in the supernatant of *P. aeruginosa* cultures using elastin congo-red assay. The anti-biofilm

activity of BAHs against *P. aeruginosa* was evaluated by crystal-violet staining, CFU count and confocal microscopy analysis. **Results:** The inhibitors CE-5 and RDL-17, when tested in culture supernatants of the reference strain PaO1, the clinical isolates PaCF1 (Cystic Fibrosis) and PaDCP2 (Primary Ciliary Dyskinesia), showed IC_{50} values against LasB activity ranging from 10 to 40 micromolar. Interestingly, CE-5 and RDL-17 maintained the ability (IC_{50}: 50-200 micromolar) to inhibit LasB when tested in diluted sputum obtained from PCD patients. RDL-17 also showed anti-biofilm activity against all three *P. aeruginosa* strains at concentrations ranging from 50 to 200 micromolar. In addition, we observed that RDL-17 increased the anti-biofilm activity of colistin against PaO1. The two inhibitors tested proved to be active in the biological assays described at non-cytotoxic concentrations. **Discussion:** Overall, these results suggest a deeper investigation into the anti-virulence capabilities of BAHs with the aim of developing new treatment strategies against *P. aeruginosa* infections. To this end, future experiments will be addressed to better characterize the activity of BAHs and to validate their anti-virulence effect in *in vivo* models of *P. aeruginosa* infection.

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175 - Human Endogenous Retrovirus K reactivation in ALS as a pathogenic factor triggering TDP-43 proteinopathy

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Human Endogenous Retrovirus K reactivation in ALS as a pathogenic factor triggering TDP-43 proteinopathy

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Introduction. Human Endogenous Retroviruses (HERVs) are relics of ancestral retroviral infections stably integrated into the human genome. Among them, HERV-K is the most recent and structurally complete, with multiple genomic copies under tight epigenetic control. In healthy cells, these elements are typically silenced. However, their reactivation from endogenous loci is increasingly associated with several pathologies, including Amyotrophic Lateral Sclerosis (ALS). Given the limitations of exogenous plasmid-based models in understanding real genome context, investigate the reactivation of endogenous HERV-K elements is essential. We observed that simulated microgravity, known to induce broad epigenetic remodeling, represents an exceptionally useful experimental model to investigate the molecular mechanisms leading to HERV-K reactivation and its pathological consequences. **Materials and Methods.** We analyzed HERV-K transcriptional levels in ALS patient-derived PBMCs and healthy controls using qPCR and measured humoral responses against HERV-K-specific epitopes via ELISA assay. Asparaginase protein levels were assessed in neuronal tissue by Western Blot, and antisense HERV-K integrations within the asparaginase gene locus were identified via USC Genome Browser and validated through PCR. SH-SY5Y neuroblastoma cells were exposed to simulated microgravity using a clinostat, and assessed

for endogenous HERV-K activation, TDP-43 pathology, and epigenetic alterations. **Results.** ALS samples displayed significantly elevated HERV-K expression relative to controls along with higher anti-HERV-K antibody titers. We observed neuronal depletion of asparaginase, associated with cytoplasmic accumulation of TDP-43. Notably, we identified antisense HERV-K insertions within the asparaginase gene, likely contributing to its transcriptional repression. While HERV-K plasmid-based overexpression allowed for exogenous expression, it did not recapitulate the activation of native loci nor the full downstream pathological features. In contrast, simulated microgravity proved highly effective in reactivating endogenous HERV-K, paralleled by epigenetic modifications. **Discussion and Conclusions.** Our results highlight HERV-K as an emerging player in ALS, likely capable of interfere with the neuroprotective processes such as TDP-43 clearance. By integrating multiple approaches such as simulated microgravity, we observed a link between retroviral reactivation, epigenetic alterations, and neurodegeneration, suggesting new targets for therapeutic approaches.

176 - The role of endogenous retrovirus mdg4 in the aggregation of hTDP-43 in Drosophila ALS model

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The role of endogenous retrovirus mdg4 in the aggregation of hTDP-43 in Drosophila ALS model

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with a fatal prognosis, characterized by the abnormal accumulation of the TDP-43 protein in motor neurons. Recent studies suggest that the activation of HERV-K (subtype HML-2), may contribute to disease pathogenesis by promoting the cytoplasmic aggregation of TDP-43. A portion of HERV-K is integrated within the genomic sequence of the Asparaginase-like-1 (ASRGL1) gene. This genomic arrangement results in potential RNA complementarity between HERV-K and ASRGL1 transcripts. ASRGL1 cleaves isoaspartates, which alter protein folding and susceptibility to proteolysis. Its downregulation triggers accumulation of misfolded, fragmented, phosphorylated and mis- localized TDP-43. In Drosophila models, activation of retroviral elements such as mdg4 (homologue of HERV-K) has also been shown to be sufficient to induce aggregation of human TDP-43, supporting a conserved pathogenic mechanism. To investigate the functional interplay among ERV-K, ASRGL1, and TARDBP (the gene encoding TDP-43), a transgenic *Drosophila melanogaster* model was generated. This line co-expresses human TARDBP (hTDP-43) and an RNA interference (RNAi) construct targeting ASRGL1, both under the control of the UAS-Gal4 system, which enables tissue-specific gene expression. **Materials and Methods**: To establish this line, UAS ASRGL1 RNAi/Sb females were crossed with UAS hTDP-43/Sb males. The resulting progeny were further crossed with TM6B/MKRS balancer lines to stabilize the genotype, yielding a hTDP-43-ASRGL1 (RNAi)/TM6B line. This line was then crossed with ELAV-Gal4/Cyo, which drives neuron-specific expression of the target constructs. Flies were reared at 25°C on a nutritional medium composed of cornmeal, agar, glucose, brewer's yeast, and Tegosept. Transgenic lines were generated through homologous

recombination, and initial screening was performed using phenotypic selection markers. Successful gene integration and protein expression were subsequently confirmed by RT-PCR and Western blot analysis. **Results**: Preliminary data indicate an increased expression of mdg4 in crosses between the line with inhibited asparaginase expression and the line expressing human TDP-43, compared to controls. This suggests a potential functional role of mdg4 in the development of the pathological phenotype. In this genetic background, asparaginase inhibition induces a phenotype characterized by motor impairment and reduced lifespan. Ongoing experiments aim to determine whether this phenotype is also associated with TDP-43 aggregation and mdg4 overexpression, potentially pointing to shared molecular pathways involved in neurodegeneration. **Discussions and Conclusions**: This experimental strategy is designed to investigate a potential feedback mechanism involving ERV-K (e.g., *mdg4* in *Drosophila*), ASRGL1, and TDP-43, supporting the hypothesis of a self-amplifying pathogenic circuit. The interaction of these factors may play a crucial role in modulating cellular stress responses, triggering pathological processes underlying neurodegenerative diseases such as ALS. The resulting transgenic line will serve as a valuable tool for dissecting these molecular interactions, providing new insights into ALS pathogenesis, and offering potential targets for therapeutic intervention.

179 - Impact of miR-9 and miR-155 on the Antiviral Response Induced by Influenza A Virus

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Impact of miR-9 and miR-155 on the Antiviral Response Induced by Influenza A Virus

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Introduction: Influenza A virus infection represents a potent stimulus for the activation of the innate immune response inducing the coordinated production of pro-inflammatory mediators and antiviral molecules. Key players in this process include inflammatory cytokines and type I interferons, which synergistically act to activate intracellular defense mechanisms and limit viral replication and spread. In this context, microRNAs (miRNAs) emerge as critical modulators of the immune response, finely regulating the gene expression of numerous inflammatory and antiviral effectors. Among these, miR-9 and miR-155 have been identified as potential regulators of these processes. This study aims to assess the role of miR-9 and miR-155 in regulating pro-inflammatory and antiviral responses by investigating the modulation of pro-inflammatory and interferon-dependent genes.

Materials and Methods: BEAS-2B cells were infected with influenza A virus H1N1 at a multiplicity of infection (MOI) of 0.1. Cell samples were collected at time points ranging from 1 to 24 hours post-infection. The expression levels of miR-9 and miR-155 were evaluated by quantitative Real-Time PCR (RT-qPCR). NF-κB activation and the expression of IL-6, TNF-α, IL-1β, IFN-β, MxA, OAS and RNase L genes or proteins were analyzed by RT-qPCR, Western blotting or ELISA.

Results: miR-9 and miR-155 expression showed a significant increase compared to controls. Concurrently, NF-κB activation exhibited a moderate upregulation associated with a significant induction of the pro-inflammatory cytokines IL-6, TNF-α, and IL-1β. A robust induction of IFN-β and MxA was also observed, indicative of a strong interferon-dependent antiviral response. OAS and RNase L expression displayed a transient kinetic pattern, peaking at 6 hours post-infection and subsequently declining.

Discussion and Conclusion: The obtained results confirm the parallel activation of the two major innate immune pathways, NF-κB-mediated inflammation and type I IFN-mediated antiviral response in an *in vitro* model of infection. The significant induction of miR-9 and miR-155, along with their temporal correlation with NF-κB activation and pro-inflammatory cytokine expression, supports their potential regulatory role in the inflammatory response. Similarly, the strong activation of the IFN-dependent response (IFN-β and MxA) suggests that these miRNAs may also modulate this arm of antiviral immunity. The limited temporal dynamics of RNase L expression may reflect lower OAS pathway activity compared to the other activated pathways. Overall, these findings support the role of miR-9 and miR-155 as regulators of innate immune responses during viral infection.

182 - Modulation of Nrf2-Associated microRNAs during Influenza A Virus Infection in Human Respiratory Epithelial Cells

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Modulation of Nrf2-Associated microRNAs during Influenza A Virus Infection in Human Respiratory Epithelial Cells

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Introduction: Respiratory virus infections have been frequently associated with the induction of oxidative stress in host cell, which in turn activates redox-sensitive pathway involved in the regulation of viral replication and inflammatory response. In particular, the modulation of NRF2-antioxidant response has been observed during influenza virus (IV) infection; however, the mechanisms underlying the virus-induced regulation have not been fully elucidated. Interestingly, some miRNAs, including miR-29c, miR-155 and miR-200 family, are involved in the maintenance of the redox balance by regulating the expression of NRF2-related genes, and their modulation contributes to the oxidative stress-induced cell damage. The aim of this study is to evaluate the expression of miRNA related to Nrf2 pathway during IV infection. Materials and Methods: Human alveolar epithelial cells (A549) or Bronchial epithelial cells (BEAS-2B) were infected with human influenza A/Puerto Rico/8/34 H1N1 (PR/8). At 4, 8 and 24 hours post infection (h.p.i.), viral replication was evaluated by TCID50 assay. Following RNA extraction,

miR-29c, miR-155 and miR-200a levels were quantified by RT-qPCR. RNU6B served as endogenous control, and relative miRNA expression was calculated as $2^{-\Delta\Delta Ct}$. Results: Viral titers increased progressively in both cell lines, from 0.9×10^2 to 2.3×10^2 in A549 and from 1×10^2 to 3.6×10^2 TCID50/ml in BEAS-2B. In A549, miRNA analysis revealed significant upregulation of miR-29c and miR-155, alongside a decrease in miR-200a levels following infection. Notably, miR-29c and miR-155 expression showed a direct correlation with viral replication, while miR-200a levels were inversely correlated. In BEAS-2B, miRNA expression exhibited a more variable trend. Deregulation of the three examined miRNAs was observed during early infection. miR29c and miR200a were over-expressed at 24 h whereas miR-155 remained upregulated at 8 h, with a significant increase observed at 24 h.p.i., corresponding with elevated viral titer. A direct correlation between miR-29c or miR-200a and viral replication was observed during early infection (≤8 h), whereas miR-155 maintained a consistent positive correlation throughout the experiment. Discussion and Conclusions: Our preliminary findings highlight a dynamic and cell-type-specific modulation of NRF2-associated miRNAs during IV infection. The observed miRNA changes may play a role in oxidative stress responses, viral replication, and inflammation. Further studies are ongoing to characterize the functional crosstalk between these miRNAs and Nrf2 signaling components across different IV strains, in order to elucidate their contribution to viral pathogenesis, especially in light of identifying potential biomarkers or novel therapeutic targets.

183 - Murine model of invasive meningococcal disease: investigating the pathogenesis of the disease

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Murine model of invasive meningococcal disease: investigating the pathogenesis of the disease

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Introduction: *Neisseria meningitidis* is a leading cause of human sepsis and meningitis worldwide. Invasive disease is preceded by asymptomatic nasopharyngeal colonization

affecting up to 18% of the normal population. In some individuals this colonizer is able to breach the mucosal barrier, get into bloodstream and multiply uncontrollably, and finally cross the blood-brain barrier to cause meningitis. Both host and bacterial factors seem to be involved in this switch from harmless transitory colonization to devastating disease. Despite significant advances in understanding the meningococcal disease's pathogenesis, progress in developing effective therapies has been limited by the lack of reliable animal models that faithfully reproduce the infection. The main aim of this study was to define the pathogenic mechanism of invasive meningococcal disease (IMD) underlying the tissue damage associated to this pathogen by using a sepsis murine model induced by intraperitoneal inoculation. **Materials and Methods:** To determine the lethal dose 50, three groups of inbred BALB/c mice (4 mice/dose) were intraperitoneally injected with *N. meningitidis* 93/4286 wild-type strain. Bacterial inocula, ranging from 10^4 to 10^6 CFU/mouse, were administered to each group. The animals were monitored for clinical signs and general condition up to 160h, recording the survival rates. To define the systemic dissemination in the murine host, three mice's groups were infected with a bacterial sub-lethal dose and euthanized at specified time points (12, 24, and 48h post-infection) for assessing viable bacterial load in the peripheral organs. Brain, spleen and liver were homogenized in 1 mL of GC broth, blood drawn by cardiac puncture was added to a tube containing 3.8% sodium citrate. Viable bacterial counts were analysed by plating 10-fold serial dilutions on GC agar plates. **Results:** Survival rates were 75% in mice infected with 10^4 and 10^5 CFU of the wild-type strain, whereas the 10^6 CFU concentration was lethal for all infected mice. A significant reduction in body weight was observed in mice infected with 10^4 and 10^5 CFU at 48h post-inoculation. However, body weight recovered slightly after 48h in the group treated with the lower bacterial dose. Bacterial loads in the organs of infected animals were consistent across time points, ranging between 10^5 and 10^6 CFU at 12 and 24h post-infection, with a slight reduction observed at 48h. **Discussion and Conclusions**: In this work, we have established a reliable murine model of meningococcal sepsis, utilizing intraperitoneal bacterial inoculation. This model may be a valuable tool for studying *N. meningitidis* pathogenesis and for the evaluation of the potential use of new adjuvant therapies to counteract IMD.

197 - Human Polyomavirus 6 differentially infects healthy and tumor colon tissue: a case report

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Human Polyomavirus 6 differentially infects healthy and tumor colon tissue: a case report

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Introduction: Human Polyomavirus 6 (HPyV6) is part of the human skin virome, but it has been detected also in blood, urine, cerebrospinal fluid, bile and cervical specimens of both immunocompetent and immunosuppressed hosts. As other HPyVs, HPyV6 encodes for two proteins with oncogenic properties, the small (sT-Ag) and large T antigens (LT-Ag). The primary asymptomatic infection occurs in childhood, then HPyV6 establishes latency in the host, with the possibility of reactivation in case of immunodeficiency.
Materials and Methods: A 73 years-old Italian female affected with right sided-colon cancer, stage pTNM II, grade 2, underwent hemicolectomy at ASST Papa Giovanni XXIII Hospital, Bergamo, Italy. The patient had previously undergone kidney transplant, and she was treated with immunuppressive therapy. The patient died after one month, because of multiresistant *P. aeuroginosa* severe sepsis. Tumor (T), adjacent healthy (AH) tissues were collected, since the patient was enrolled, after signing informed consent, in a study aimed to verify the possible association between virus and colon cancer, previously approved by the local ethical committee. Next Genomic Sequencing was conducted on the extracted total nucleic acids, using Illumina platform, followed by viral metagenomics analysis. HPyVs detection was confirmed by Multiplex Real-Time PCR and PCR targeting the viral Non-Coding Control Region (NCCR), the Viral Protein 1 (VP1) and the LT-Ag encoding genes.
Results: Viral metagenomics analysis revealed that 507 (0.45%), and 470 (0.30%) different viral genomes infected the AH and T tissues, respectively. Both AH and T tissues mainly harboured

viral unclassified or phages genomic sequences; the apathogenic Henipavirus Cedar and the vesiculovirus gorganvirus isfahan were found at same percentages in both AH and T tissues. Interestingly, HPyV6 was detected in T, but not in AH tissue, accounting for 7% of all the viral sequences. The subsequent molecular analysis confirmed the presence of high load of HPyV6 genome ($2.4x10^9$ copies/ug), infecting high percentage ($1.5x10^4$%) of T tissue cells. HPyV6 NCCR, LT-Ag, and VP1were all detected in the T tissue. No other HPyVs was present.
Conclusions: We reported the first detection of HPyV6 in the colon tissue, with differential infection of the tumor rather than the healthy adjacent tissue in a colon cancer patient. The high viral load and percentage of infected cells might be indicative of an ongoing active replication of the HPyV6, that could have been induced by the immunosuppression status of the patient. As for other HPyVs, HPyV6 might play a triggering role in the colon malignant transformation.

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214 - Induction of the unfolded protein response during Toscana virus and Sandfly Fever Naples virus infection

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Introduction. Phleboviruses (*Hareavirales* class; *Phenuiviridae* family; *Phlebovirus* genus) are single-stranded, tri-segmented RNA genome viruses, transmitted by sandflies, and isolated worldwide. Toscana virus (TOSV) and Sandfly Fever Naples virus (SFNV) are the most common phleboviruses isolated in the Mediterranean basin. Despite they are antigenically related, TOSV can infect the central and peripheral nervous system, with a potentially fatal outcome, whereas SFNV usually leads to transient and self-limiting, flu-like symptoms (sandfly fever). So far, no vaccine and/or pharmacological treatment are available to treat phleboviral infections. Hence, the comprehension of their replicative cycle and the host response may provide some targets for new bioactive compounds. One of the first defenses is the unfolded protein response (UPR), which can increase the endoplasmic reticulum folding capacity and block the translation process, leading to apoptosis if the injury cannot be managed. On the other hand, viruses need

an efficient translational machinery to produce their proteins, necessary for the viral progeny assembly; thus, they have developed strategies to hijack for their benefit, positively or negatively, the UPR. In this context, the current study has focused on the UPR during an *in vitro* TOSV and SFNV infection model. **Materials and Methods.** The experiments were set up using different multiplicities of infection (MOI, high MOI: 3; low MOI: 0.1) and harvesting at different times post-infection (from 0 to 18 hours). The RNA content of infected- and mock-infected human lung cells (A549) was defined by RNA-sequencing (RNA-seq) technique and then analyzed by bioinformatic tools. In the meanwhile, the samples underwent quantitative real-time PCR (qPCR) and Western Blot analyses to detect the potential modulation of UPR upon infection. **Results.** Our results have revealed that UPR is modulated upon infection, with its main effectors (BIP/GRP78, PERK, ATF6, IRE1 and CHOP) registering a strong upregulation in the late phase of infection. **Discussion and Conclusions.** Our preliminary findings highlight a previously unrecognized role of the UPR in phlebovirus infection and suggest that both TOSV and SFNV actively engage and manipulate this response during their replication cycles. The late-phase upregulation of key UPR effectors may reflect a viral strategy to optimize replication or evade host defenses. This study opens potential avenues for targeting the UPR pathway in antiviral strategies against phleboviral infections.

216 - Induction of the unfolded protein response during Toscana virus and Sandfly Fever Naples virus infection

Francesca Palma *(1)* ***- Bianca Maria Nastri*** *(1)* ***- Laura Di Clemente*** *(1)* ***- Carla Capasso*** *(1)* ***- Simone La Frazia*** *(2)* ***- Valentina Svicher*** *(2)* ***- Carla Zannella*** *(1)* ***- Anna De Filippis*** *(1)* ***- Massimiliano Galdiero*** *(1)*

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an efficient translational machinery to produce their proteins, necessary for the viral progeny assembly; thus, they have developed strategies to hijack for their benefit, positively or negatively, the UPR. In this context, the current study has focused on the UPR during an *in vitro* TOSV and SFNV infection model. **Materials and Methods.** The experiments were set up using different multiplicities of infection (MOI, high MOI: 3; low MOI: 0.1) and harvesting at different times post-infection (from 0 to 18 hours). The RNA content of infected- and mock-infected human lung cells (A549) was defined by RNA-sequencing (RNA-seq) technique and then analyzed by bioinformatic tools. In the meanwhile, the samples underwent quantitative real-time PCR (qPCR) and Western Blot analyses to detect the potential modulation of UPR upon infection. **Results.** Our results have revealed that UPR is modulated upon infection, with its main effectors (BIP/GRP78, PERK, ATF6, IRE1 and CHOP) registering a strong upregulation in the late phase of infection. **Discussion and Conclusions.** Our preliminary findings highlight a previously unrecognized role of the UPR in phlebovirus infection and suggest that both TOSV and SFNV actively engage and manipulate this response during their replication cycles. The late-phase upregulation of key UPR effectors may reflect a viral strategy to optimize replication or evade host defenses. This study opens potential avenues for targeting the UPR pathway in antiviral strategies against phleboviral infections.

225 - Unlocking Invasive Salmonella enterica Serotypes: Insights from a 15-Year Regional Biobank (2001–2016)

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Unlocking Invasive *Salmonella enterica* Serotypes: Insights from a 15-Year Regional Biobank (2001–2016)

Background. Salmonellosis is the second most commonly reported food-borne zoonosis in the European Union (ECDC, 2023) and globally causing to 80 million diarrheal cases each year. Non-typhoidal *Salmonella* (NTS) primarily cause self-limiting gastroenteritis, though some serotypes can lead to extra-intestinal infections and bacteremia. We analyzed clinical strains of *S. enterica* isolated in Lombardy Region since 2001 to 2016, preserved in a biobank in our laboratory. Every sample was accompanied by a datasheet with epidemiological, clinical and demographical data corresponding to the related salmonellosis case.

Materials and Methods. In the biobank we found 6.624 clinical *S. enterica* strains isolated in the period 2001-2016 from salmonellosis cases and serotyped according to the Kauffman-White classification scheme. We analyzed the data reported in the archive focusing on invasive serotypes of the biobank by comparing growth curve and fitness of different bloodstream serotypes.

Results. A total of 172 NTS were identified. The most prevalent NTS serotypes were *S.* Typhimurium (41.8%, n=2.772), *S.* Enteritidis (18.5%, n=1.223), *S.* 1,4,[5],12:i:- (9.8%, n=649), *S.* Napoli (9.6%, n=636). The highest proportion of cases occurred in children aged 0-4 years (37.7%, n=2.213 p<0.0001). Bloodstream infections were found in 244 patients, with *S.* Choleraesuis, *S.* Typhimurium and *S.* Napoli being the three most common serotypes (24.6%, n=60; 22.5%, n=55; 15.8%, n=38). *S.* Choleraesuis showed an Invasive Index (77%, 60/78) higher than *S.* Typhi (64.7%, 22/34, p<0.0001) and the higher rate of hospitalization observed

(92.1% 58/63 *vs* 42%, 1.821/4.345; *S*. Choleraesuis *vs* all NTS, p<0.0001). Comparing the growth curve of *S*. Choleraesuis and *S*. Typhimurium strains, both isolated from bloodstream, a disadvantage in the growth rate and fitness was observed in *S*. Choleraesuis.

Discussion and Conclusions. Despite the high burden of salmonellosis, there is a lack of information to monitor this disease over time. Although our data looked only at the tip of the iceberg, the analysis of our historical archive and of the biobank was useful to reconstruct epidemiological trend of salmonellosis and the characteristic of the past circulating strains. We focused on serotypes which showed a high Invasive Index and performed fitness experiments. The observed slow-growing rate of *S*. Choleraesuis strains may suggest the harbor of a larger genome or virulence plasmids, conferring enhanced invasiveness and resistance mechanisms. Further studies will be needed to investigate the molecular invasiveness mechanisms of Salmonella, in particular *S*. Choleraesuis and *S*. Napoli, which have a common ancestor, the one with *S*. Paratyphi *C* and the other with *S*. Paratyphi *A*.

226 - Activity of Ranalexin-1G against Pseudomonas aeruginosa, a leading cause of lung infections in patients with cystic fibrosis.

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Activity of Ranalexin-1G against *Pseudomonas aeruginosa*, a leading cause of lung infections in patients with cystic fibrosis.

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Introduction. Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene, which encodes an anion channel responsible for transporting chloride and bicarbonate across the apical membranes of epithelial cells. Bicarbonate ions play a vital role in the hydration and unfolding of mucins, particularly in the airways. These changes impair bacterial clearance and contribute to chronic respiratory infections, primarily caused by *Pseudomonas aeruginosa* (*Pa*).Treating lung infections associated with CF is challenging due to the presence of multidrug-resistant *P. aeruginosa*, capable of forming biofilms and triggering inflammation, ultimately resulting in respiratory complications. Antimicrobial peptides (AMPs) are crucial components found in all living organisms, and they exhibit antimicrobial activity. Our study aimed to evaluate antibacterial activity of Ranalexin-1G, an antimicrobial peptide (AMP) from the skin of the Rana grylio, against *Paeruginosa*. Materials and Methods. The cytotoxicity profile of Ranalexin-1G was analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay on HacaT cell line (human keratinocytes) in the range of concentrations from 100 to 0,7 μg/mL. Antibacterial activity against *P. aeruginosa* ATCC 13388 was evaluated by broth microdilution method and the crystal violet (CV) staining. A time-kill assay was also performed by quantifying colony-forming units (CFUs) at different times. In addition, some virulence factor genes expression via RT-PCR was analized to assess the peptide anti-bacterial effect. Finally, to evaluate whether Renalexin on *Pa* invasion capability, we perform invasion assays using human lung epithelial cell model (BEAS-2B). Results. Ranalexin-1G showed a low cytotoxicity, with

90% cell viability at 50 µg/mL. MIC value for the peptide was 25 µg/mL, demonstrating a bactericidal effect after 3 hours. The expression of virulence factor genes, including alginate, protease, and exotoxins was been evaluated, highlighting gene expression modulation. In addition, Ranalexin-1G antimicrobial activity was demonstrated by inhibition of *Pa* cell invasion. Discussion and Conclusions. To our knowledge, this is the first study examining the antimicrobial properties of Ranalexin-1G in a cellular model infected with *Pseudomonas aeruginosa*. Our findings highlight its ability to modulate virulence factors, making it a promising candidate for treating lung infections in patients with cystic fibrosis.

227 - Fusobacterium nucleatum promotes subspecies-and strain- specific cancer cell proliferation

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Fusobacterium nucleatum promotes subspecies- and strain-specific Cancer Cell Proliferation

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Introduction: Accumulating evidence shows that the common inhabitant of the oral cavity, *Fusobacterium nucleatum (Fn)* is involved in the progression of an increasing number of tumor types. The mechanisms underlying tumor exacerbation by *Fn* include enhancement of cell proliferation, metastasis, establishment of a tumor-promoting immune environment, induction of chemoresistance and the activation of immune checkpoints. Notably, the presence of multiple subspecies of *Fn,* each contributing differently to these processes, has been highlighted.

The aim of this study was to characterize different subspecies and strains of *Fn* according to their ability to promote cancer cells proliferation and to activate key pathway involved in tumorigeneses.

Materials and Methods: Fusobacteria were isolated from clinical specimens, identified using MALDI-TOF and genetic analysis. Infection experiments were performed using Caco-2 (colorectal adenocarcinoma), HN-13 (head and neck carcinoma) and MCF-7 (breast cancer) cell lines with *Fn* ATCC 25586 and a set of strains belonging to the subspecies nucleatum, animalis, polymorphum, and vincentii, isolated from OSCC (oral squamous cell carcinoma) patients. A GFP-expressing Fn ATCC strain was generated by cloning the pVopO-GFP plasmid. Cell proliferation was evaluated by nuclear staining and automated cell counting using the Cytation system. Intracellular persistence was assessed by CFU counts. Cell migration and proliferation were also evaluated by wound healing (scratch) assays. To study macrophage interactions, THP-1 cells were infected, and bacterial persistence was measured. Activation of immune pathways was assessed by infecting THP-1 xBlue (Invivogen) and HEK INF-alpha/beta (Invivogen) reporter cell lines to measure NF-κB and type I interferon (INF-1) expression.

Result: We successfully generated a fluorescent GFP-expressing *Fn* ssp *nucleatum* ATCC strain and defined the experimental conditions to assess the biological effects using Caco-2 cells: we used different MOI, with the presence or not of antibiotics during the incubation period and measured intracellular and extracellular CFUs. A significant cell proliferation was measured 24h post-infection.

Subsequent infections of Caco-2, HN-13, and MCF-7 cell lines with 37 clinical Fusobacterium strains revealed that *Fn subsp. animalis* and *Fn subsp. nucleatum* were the most potent in promoting cancer cell proliferation, as demonstrated by increased cell counts, scratch assays, and invasion assays.

Infection of THP-1 cells at different MOIs confirmed the persistence of *Fn* within macrophages after 24 hours. Furthermore, stimulation of THP-1 xBlue and HEK INF-alpha/beta cells with different *Fn* subspecies showed varying degrees of NF-κB and type I interferon activation.

Discussion and Conclusion: Genetic and molecular characterization of these Fusobacterium strains, along with the identification of host cell pathways affected during infection will help us unravel the impact of bacterial variability in the tumorigenic properties of this emerging bacterial species.

228 - The use of Resveratrol Conjugated with Nanoparticles Against H.Pylory Infection

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The Use of Resveratrol Conjugated with Nanoparticles Against *H. pylori* Infection

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Introduction: *Helicobacter pylori* is a microaerophilic, Gram-negative, spiral-shaped bacterium capable of colonizing the stomach. Several virulence mechanisms have been identified, including the production of urease, the gene products of the pathogenicity island (cagPAI – *cag* pathogenicity island), and the vacuolating cytotoxin (*vacA*). The ability to adhere to surfaces and form biofilms are also critical virulence factors that hinder effective drug therapy. Trans-resveratrol (RESV), a bioactive polyphenol derived from natural sources, has shown potential activity against this gastric pathogen.
The aim of our study was to analyze the activity of RESV conjugated with nanoparticles against *H. pylori* biofilm formation.

Materials and Methods: The study was carried out on the *H. pylori* ATCC 26695 strain under static conditions using the crystal violet staining method.
RESV was tested at increasing concentrations (equivalent to 2, 4, 8, 16, and 32 µg/mL). All in vitro assays were performed in duplicate.

Results:Interestingly, the RESV-conjugated nanoparticles exhibited intrinsic antibiofilm properties, which were further enhanced by the presence of the active substance. A concentration-dependent effect was observed.
As a result, the proposed nanoparticles appear to be effective at extremely low doses in achieving antibiofilm effects.

Discussion and Conclusions: The results obtained in this study demonstrate the antibacterial activity of RESV-conjugated nanoparticles against *H. pylori* ATCC strain, providing valuable

insights into their potential use in combination with antibiotics to improve eradication efficacy. This finding is encouraging for the development of new therapeutic strategies involving natural products such as RESV as alternative approache to treat *H. pylori* infections.

230 - Development of an in vitro model of DNase-resistant Methicillin-Resistant Staphylococcus aureus (MRSA) biofilm reveals Z-DNA enrichment

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Development of an *in vitro* model of DNase-resistant Methicillin-Resistant *Staphylococcus aureus* (MRSA) biofilm reveals Z-DNA enrichment

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Introduction
Staphylococcus aureus is a common commensal responsible for a wide range of infections, including skin and soft tissue infections, bacteremia, osteomyelitis, pneumonia, prosthetic device infections, and endocarditis. Its ability to form robust biofilms is a key factor contributing to chronic infections and antibiotic resistance. Extracellular DNA (eDNA) is a key structural component of the biofilm matrix. Recent studies suggest that eDNA may adopt alternative conformations, including Z-DNA, a left-handed helix known for its resistance to enzymatic degradation. The aim of this study was to develop an *in vitro* model of *S. aureus* biofilm to evaluate DNase resistance and investigate the presence of Z-DNA within the biofilm matrix.
Materials and Methods
To assess biofilm formation, the methicillin-resistant *S. aureus* (MRSA) reference strain ATCC 43300 was incubated at 37 °C and 150 rpm for 24, 48, and 72 hours, with medium replacement every 24 hours. Various media were tested: TSB, TSB supplemented with 5 µM hemin, 1% NaCl, 1% glucose, and their combinations. Biofilm biomass and metabolic activity were quantified using crystal violet staining and the XTT reduction assay, respectively. To evaluate extracellular DNA (eDNA) susceptibility to enzymatic degradation, preformed biofilms were treated with DNase I for 3 hours, and biomass was measured as described above. Z-DNA and B-DNA

detection within the biofilm matrix was analyzed using confocal laser scanning microscopy (CLSM).

Results

Our findings showed that MRSA was able to form a strong and stable biofilm between 24 and 72 hours, when cultured in TSB supplemented with 1% glucose, 1% glucose plus 5 µM hemin, or 1% glucose plus 5 µM hemin and 1% NaCl. DNase I treatment revealed that the biofilm biomass formed in TSB containing 1% glucose and 5 µM hemin exhibited the greatest resistance to enzymatic degradation. CLSM analysis of 48-hour-old biofilms grown under this condition showed a Z-DNA/B-DNA fluorescence ratio greater than 1, indicating a predominance of Z-DNA over B-DNA within the biofilm matrix.

Discussion and conclusions

The present study provides the first evidence of Z-DNA presence within MRSA biofilm in an in vitro model. Under specific growth conditions that promote biofilm stability and DNase I resistance, we observed a clear predominance of Z-DNA over B-DNA, suggesting a potential contribution to MRSA biofilm integrity. By establishing an *in vitro* model of MRSA biofilm DNase resistant enriched with Z-DNA, this study offers a valuable tool for exploring Z-DNA function in biofilm architecture and its potential as a target for antibiofilm strategies.

233 - Disserting the activity of Cell Free Supernatant of Lactobacillus crispatus M247: a promising treatment against vaginal infections

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Disserting the activity of Cell Free Supernatant of *Lactobacillus crispatus* M247: a promising treatment against vaginal infections

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Introduction

Lactobacillus crispatus is renowned for its antimicrobial properties and some strains are used to treat vaginal dysbiosis, though the mechanisms underlying the antimicrobial properties remain elusive. Notably, *L. crispatus* is one of the dominant species found in the vaginal microbiota of healthy, reproductive-age women and is strongly associated with eubiotic conditions. Its presence is often correlated with a low vaginal pH, a reduced risk of bacterial vaginosis, and protection against sexually transmitted infections, highlighting its potential role as a key biomarker and contributor to vaginal health.

Materials and Methods

We isolated *L. crispatus* M247 (LcM247) from the commercially available probiotic Crispact® its antimicrobial activity was assessed against selected pathobionts such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Enterococcus faecalis* and *Candida albicans* by testing the bacterium and its cell-free supernatant (CFS). To elucidate the antimicrobial activity, CFS was: a) serially diluted, b) pH of the suspension was alkalinized and c) the suspension was heat inactivated or treated with proteinase K. Finally, we set up a series of *in vitro* infection models based on Henrietta Lacks' cervical eukaryotic cancer

cells (HeLa) which were infected with *E. coli*, *S. agalactiae* and *C. albicans* before administration of LcM247, CFS, antimicrobial compounds alone or in combination with LcM247/CFS.

Results

In axenic culture, we measured that *L. crispatus* determined minimal inhibition of pathogen growth following 4 hours of incubation, while CFS significantly reduced bacterial growth. Conversely, both LcM247and its CFS showed comparable bactericidal effects 24 hours post incubation against all indicator strains except for *E. faecalis* and *C. albicans*. Interestingly, increasing the pH of the supernatant decreased the CFS antimicrobial activity, suggesting that its antimicrobial activity is pH dependent. No other modifications impaired CFS activity. When HeLa were treated following infection, CFS showed a superior antimicrobial efficacy compared to antimicrobial compounds or to LcM247 alone. Additionally, a synergistic effect was measured when the combination of CFS-antimicrobial compounds was administrated.

Discussion and conclusions

These results confirm the role of LcM247 in the control of some indicator strains, highlighting the promising activity of its CFS as a potential natural antimicrobial agent.

Indeed, we evidence that actively replicating LcM247 is less efficient than its CFS, so that the administration of LcM247 alone may result in a treatment failure. The use of CFS may trigger the upswing of the host *Lactobacillus* strains or promoting the engraftment of *Lactobacillus* probiotic treatments.

236 - Intra-hepatic HDV replicative activity can be sustained by HBsAg production from HBV integration and can modulate virological response to bulevirtide

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Intra-hepatic HDV replicative activity can be sustained by HBsAg production from HBV integration and can modulate virological response to bulevirtide

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Introduction: The contribution of Hepatitis B virus (HBV) integration in sustaining Hepatitis D virus (HDV) activity by providing surface proteins (HBsAg) is still not well elucidated. Furthermore, the potential role of intrahepatic viral markers and transcriptome profiles in modulating response to bulevirtide (BLV) deserves investigation. **Materials and Methods:** We analyzed 70 liver biopsies from 35 patients (pts) with chronic hepatitis B (CHB) and 35 with chronic hepatitis D (CHD), 6 of them completing 24 weeks of BLV. Combined response to BLV was defined as >2log HDV-RNA decay plus transaminases normalization. Droplet-digital PCR was used to quantify intrahepatic levels of covalently closed circular DNA (cccDNA), pregenomic RNA (pgRNA), HDV-RNA and HBsAg transcripts from cccDNA and from integrated HBV-DNA. HBsAg isoforms (small [S-], middle[M-] and large[L-HBsAg] HBsAg) were quantified by ELISAs. RNA-Seq by Illumina was applied for transcriptome analysis. **Results:** CHD was characterized by lower cccDNA and pgRNA than CHB (median[IQR]: 1[0.02-12] vs 24[8-93]copies(cps)/1000cells and 8[1-147] vs 518[57-3,894]cps/1000cells, P<0.0001 for both). Despite this, in both groups, a considerable production of HBsAg transcripts was observed with >99% of them from integrated HBV-DNA (median[IQR]: 6,041[323-29,446] and 12,776[4,570-55,977]cps/1000cells). In CHD, no correlation was observed between cccDNA and intrahepatic HDV-RNA (Rho=0.1, P=0.6). Notably, 8 pts, despite undetectable cccDNA, cccDNA-derived HBsAg transcripts and serum HBV-DNA, had considerable levels of intrahepatic and serum HDV-RNA (median[IQR]: 5,495[976-14,946]cps/1000 cells and 6.0[4.8-6.9]logIU/ml), as well as of integration-derived HBsAg transcripts (median[IQR]: 3[1-497]cps/1000 cells) and of all three HBsAg isoforms (median[IQR]ng/ml: 1,116[123-3,987] for S-, 368[8-1,894] for M- and 1.4[0.2-5.6]ng/ml for L-HBsAg). Focusing on 6 CHD pts undergoing BLV therapy, 3 achieved combined response at week 12 and 3 at week 24. At baseline, the levels of intrahepatic HDV-RNA and integration-derived HBsAg transcripts tended to be lower in responders at week 12 (median[IQR]: 2,262[1,777-2,913] vs 8,760[4,656-9,046] for HDV-RNA and 12,770[6,730-22,421] vs 55,417[30,729-56,982]cps/1000cells for integration-derived

HBsAg transcripts). Notably, the immune checkpoint TIGIT tended to be up-regulated in earlier responders (log2 fold-change: 1.45; adjusted P=0.04). **Discussion and Conclusions:** HDV persistence is independent from the intrahepatic HBV reservoir and is sustained by HBsAg derived from integrated HBV-DNA. Moreover, a reduced intrahepatic HDV reservoir and limited HBV integration, as well as a more finely modulated immune system, may correlate with a rapid control of HDV replication under BLV.

247 - Study of the endothelial response to SARS-CoV-2 in vitro infection of human lung microvascular endothelial cells

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Study of the endothelial response to SARS-CoV-2 *in vitro* infection of human lung microvascular endothelial cells

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Background: Infectious agents can damage vascular endothelium causing vascular diseases. SARS-CoV-2 infection has been strongly associated with overproduction of a wide range of proinflammatory mediators, causing critical illness. Despite many clinical evidences of a link between endothelial inflammatory impairment and SARS-CoV-2 infection, the mechanism underling this link remains unclear. The aim of the project was to investigate the correlation between SARS-CoV-2 infection and endothelial disfunction in an *in vitro* model of human lung microvascular endothelial cells (HULEC-5a).

Methods: HULEC-5a cell line was infected with three different SARS-CoV-2 lineages (B.1, B.1.617.2 and B.1.1.529, BA.1). Cells and their medium were collected at different timepoints post-infection (p.i.) (from 2h to 6 days p.i.) and analyzed by the means of quantitative real time PCR and plaque assays to define the kinetic of infection. A panel of cellular inflammatory and adhesion molecules transcripts was analyzed by the means of real time PCR. Nitric oxide pathway was studied by analyzing transcriptional and proteomic expression of endothelial nitric oxide synthases (NOS) coupled to NO production.

Results: HULEC-5a resulted susceptible to SARS-CoV-2 infection showing the presence of the virus in both cells and medium collected at all the timepoints p.i. Permissibility of the cells was observed only for the B.1.617.2 lineage. Inflammatory transcripts analysis showed a significant increase in the transcript levels of pro-inflammatory TNFα and IL1β at 6h p.i. with B.1 and B.1.617.2 lineages respectively, and a significant increase in the transcript levels of the anti-inflammatory IFNβ at 24h and 72h p.i. after infection with the B.1.1.529, BA.1. Adhesion molecules transcripts showed different increase pathways among SARS-CoV-2 lineages. The NO pathway seems to be post transcriptional regulated.

Conclusions: To our knowledge, this is the first demonstration of the capability of lung endothelial cells to be directly infected with SARS-CoV-2 different lineages. The increase of pro-inflammatory and adhesion molecules transcripts after infection might be linked to cytokine overproduction, as experienced with infections with B.1 and B.1.617.2 lineages. Downregulation of the NO pathway could get worse the pro-inflammatory status.

249 - Survival at all costs: Genetic drivers of Staphylococcus aureus in bloodstream

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Survival at all costs: Genetic drivers of *Staphylococcus aureus* in bloodstream

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Introduction: *Staphylococcus aureus (S. aureus)* bloodstream infections are a major clinical concern due to the pathogen's ability to adapt to diverse host environments and evade immune responses. A deeper understanding of the genetic determinants that underlie survival in blood is critical for identifying novel therapeutic targets. This study aims to define both fitness-enhancing and fitness-reducing genes that influence *S. aureus* survival in human blood.

Materials and Methods: A genome-wide screen of a highly saturated methicillin-resistant *S. aureus* (MRSA) transposon mutant library was conducted using Transposon-Directed Insertion Site Sequencing (TraDIS) following exposure to human blood. Sequencing data were analyzed using the BioTraDIS pipeline to identify both underrepresented and overrepresented mutants. Genes of interest, representing major functional pathways, were individually validated using fitness assays. Comparative genomic analysis was performed across differennt MRSA lineages to assess conservation and strain-specific adaptation.

Results: Our TraDIS screen identified a set of conserved genes fundamental for *S. aureus* survival in human blood. These included genes involved in purine biosynthesis, electron transport, metal ion acquisition, and heme detoxification. Disruption of key genes in these pathways led to a significant reduction in bacterial survival. Notably, mutants lacking the global

regulators, particularly those affecting surface protein expression, exhibited enhanced survival in blood, suggesting a trade-off between virulence and fitness under bloodstream conditions. Comparative analysis revealed that while many fitness-related genes were conserved across MRSA lineages, others were strain-specific, indicating both universal and lineage-specific adaptation strategies.

Conclusion: This study provides a comprehensive genome-scale view of the genetic requirements for *S. aureus* survival in human blood, revealing a network of metabolic and regulatory genes essential for bloodstream fitness. The observed inverse relationship between virulence regulation and survival highlights an adaptive strategy that prioritizes persistence over toxicity. These findings enhance our understanding of *S. aureus* pathogenesis during invasive infection and offer a valuable framework for identifying therapeutic targets aimed at disrupting bacterial fitness in the bloodstream.

252 - Pantinin-1: an antimicrobial peptide effective against Acinetobacter baumannii infection.

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Pantinin-1: an antimicrobial peptide effective against *Acinetobacter baumannii* infection.

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Introduction. The rise of antimicrobial resistance is one of the most serious threats to global public health. This has led to selection of resistant micro-organisms and contributed to the emergence of so-called 'super-bacteria'. Among these is *Acinetobacter baumannii*, a Gram-negative bacterium responsible for often serious and difficult-to-treat nosocomial infections. It has become a global priority due to its significant ability to develop multiple resistance to available antibiotics. In this context, antimicrobial peptides (AMPs), which are natural molecules produced by various organisms, are emerging as promising alternatives to conventional antibiotics. The aim of this study was to evaluate the antibacterial activity of the peptide Pantinin-1, isolated from the venom of the scorpion *Pandinus imperator*, against *A. baumannii*. The study analyzed its effect on different *Acinetobacter* virulence factor Materials and Methods. The cytotoxicity of the peptide was assessed on the HaCaT cell line (human keratinocytes) by MTT method. Antibacterial activity was tested against *Acinetobacter baumannii* ATCC and a clinical strain Class D carbapenemase producer by liquid-phase microdilution assay to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A time-kill test was carried out to evaluate the colony forming units (CFU) at different times, while peptide antibiofilm action was evaluated by the violet crystal staining method, In addition, the effect of the peptides on the gene expression of *A. baumannii* virulence factors was assessed at three different time by the PCR method.

Results. The MTT test revealed that the non-cytotoxic concentration of Pantinin-1 was 50 μM. This peptide showed a remarkable effect against A. baumannii, with a bactericidal effect already after 1 hour against both the ATCC strain and the clinical isolate, with MICs at 6.25 μM for both. The peptide showed good action against biofilm, in detail against attachment phase, with a reduction of around 40% at the MIC value. Finally, the expression of genes encoding virulence factors such as biofilm production and cell adhesion was reduced by up to 40-50% after treatment, with better results after 2.5 and 24 hours. Discussion and Conclusions. Our study showed that Pantinin-1 can be considered a promising innovative antimicrobial peptide, showing its therapeutic activity against multidrug-resistant bacterial pathogens.

253 - Peptides from natural sources to counteract antimicrobial resistance through innovative delivery systems.

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Peptides from natural sources to counteract antimicrobial resistance through innovative delivery systems.

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Project Objectives. The aim of the project is to identify a library of antimicrobial peptides (AMPs), derived from different natural sources and conjugate them with hydrogels, polymeric materials with a porous net structure that can be loaded with antimicrobial peptides, natural substances and antibiotics. The AMPs are part of the innate immune system of many organisms and show activity against a wide range of bacterial, fungal and viral pathogens. It becomes necessary to search for alternative antimicrobial agents in view of the growing threat of antimicrobial resistance. Hydrogels have excellent biological activity and are biodegradable, promising ideal wound treatments. These innovative delivery systems are of low toxicity and allow controlled slow release for the treatment of wound infections. Scientific Rational. The skin represents a defence barrier against pathogens, so when it loses its integrity it is susceptible to colonisation by pathogens. Among these, the ESKAPEE pathogens are a group of multidrug-restitant (MDR) bacteria, which are particularly targeted. This group is involved in many nosocomial infections, in particular it is responsible for skin and soft tissue infections (SSTI). The colonisation of wounds by these bacteria can cause bacterial infections of the circulatory system, even leading to death. The expected results of this research project are therefore the formulation of hydrogels loaded with different antimicrobial peptides (AMPs)

whose antibacterial power will be tested against both ATCC bacterial strains and clinical isolates, also in combination with antibiotics, for wound repair and healing. Preliminary experimental data. We started by testing three peptides: Pantinin-1 derived from the venom of the scorpion Pandinus imperator, Ranalexin-1G derived from the skin secretions of the frog American bullfrog and Pseudin-2 derived from the skin secretions of the frog Pseudis paradoxa. The MTT test revealed that the safe concentration on was 50 μM for Pantinin-1 and Pseudin-2 while for Ranalexin-1G it was 25 μM. All peptides had a remarkable antibacterial action against *Acinetobacter baumannii* in a concentration range of 12.5 μM to 6.25 μM. The three peptides had a bactericidal effect: Ranlexin-1G after six hours, Pseudin-2 after three hours and Pantinin-1 after one hour. The evaluation was also extended to clinical isolates showing some resistance, such as *A. baumannii* producing carbapenemase class D. Results were noteworthy with MICs recorded in the range from 12.5 μM to 6.25 μM. In addition, the expression of genes responsible for virulence factors, such as biofilm production and cell adhesion, was reduced by 40-50% after treatment, indicating promising prospects against other ESKAPEE bacteria. Subsequent studies will evaluate the effectiveness of these peptides in wound healing through in vitro analysis of wound repair. This process will also be examined at a molecular level by analyzing factors involved in wound healing, such as metalloproteinases, while exploring the potential of conjugating peptides with delivery systems like hydrogels.

258 - Survive to Thrive: Mapping the Genetic Blueprint of S. aureus Survival in vivo

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Survive to Thrive: Mapping the Genetic Blueprint of S. aureus Survival *in vivo*.

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Introduction

Staphylococcus aureus is a versatile pathogen capable of adapting to a wide range of host environments. However, the genetic determinants that enable this adaptability during infection remain only partially understood.

Materials and Methods

We applied Transposon-Directed Insertion Site Sequencing (TraDIS) to a highly saturated mutant library of *S. aureus* USA300 JE2 to identify fitness genes in murine models of skin and systemic infection. To validate key hits, in vivo competition assays were performed using selected mutants from the Nebraska Transposon Mutant Library.

Results

The analysis revealed both shared and niche-specific genetic requirements for *S.aureus* persistence. Certain genes were consistently associated with in vivo fitness across models,

while others exhibited organ-specific depletion patterns. Functional assays supported the differential importance of selected genes in defined infection contexts.

Discussion and Conclusions

This study highlights the tissue-dependent nature of *S. aureus* gene essentiality during infection. The findings provide a framework for understanding how pathogens adjust their genetic programs to meet diverse host challenges and may inform future studies into microbial adaptation and intervention strategies**.**

269 - Frog-skin derived peptide: promising strategy against carbapenem-resistant Escherichia coli and Klebsiella pneumoniae Infections

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Frog-skin derived peptide: promising strategy against carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* Infections

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Introduction. The continuous outbreak of drug-resistant bacterial infections imposes the urgent need to search for new drug candidates. *Klebsiella pneumoniae* (*K. pneumoniae) and Escherichia coli* infections are widespread in hospitals and particularly dangerous to human health. In recent years, an increase of carbapenem-resistant *Enterobacteriaceae,* has been observed determining respiratory and urinary tract affections. In this context, antimicrobial peptides (AMPs) have gained significant attention as a promising alternative to conventional drugs, owing to their antimicrobial potency, low toxicity, and reduced propensity for fostering resistance. Our research aims to investigate the antibacterial ability of three amphibian AMPs, namely Hylin-a1, AR-23, and RV-23, against both antibiotic-sensitive and carbapenem-resistant strains of *Escherichia coli* and *Klebsiella pneumoniae*. **Material and Methods.** A 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) was performed to

identify non-cytotoxic concentrations of peptides. A microdilution assay evaluated the antibacterial effect, determining the peptides' minimum inhibitory concentration (MIC). Antibacterial activity was performed against *K. pneumoniae* ATCC 10031, E.coli ATCC and several carbapenem-resistant clinical isolates. In addition, a time-kill test was performed by recording the colonies forming units (CFUs) at several times, and compared with those of the untreated bacteria. Finally, the checkerboard test analyzed the compounds' synergistic effect with antibiotic. **Results**. We demonstrated that peptides with low toxicity profile and resistance to proteolytic activity exhibited strong antibacterial activity, with MIC ranging from 6.25 to 25 μM. The antibiofilm mechanism of action of peptides was also investigated, suggesting that they had a crucial role during the biofilm formation step by inhibiting it. Finally, we highlighted the synergistic effects of peptides with meropenem. **Discussion and conclusions.** Our study identifies Hylin-a1, AR-23, and RV-23 as promising candidates against Gram-negative bacterial infections with a favorable therapeutic profile. This effect could be related to their great flexibility, as evidenced by circular dichroism data, confirming that the peptides could assume an α-helical conformation interacting with bacterial membranes.

276 - Interkingdom interactions between Candida albicans and Enterococcus faecalis in dual-species biofilms

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Interkingdom interactions between *Candida albicans* and *Enterococcus faecalis* in dual-species biofilms

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Introduction

Candida albicans and *Enterococcus faecalis* are opportunistic pathogens that may be co-isolated from various human body niches, including the oral cavity. Their co-occurrence in biofilm-related infections suggests a potential interaction, though the nature of their relationship remains unclear. Previous reports describe conflicting outcomes, ranging from synergy to antagonism, highlighting the complexity of the interaction. Bacterial presence and metabolites can modulate *C. albicans,* yeast presence also affecting bacterial virulence. Given their shared niches and clinical significance, a deeper investigation into interaction dynamics is advisable.

Materials and Methods

In vitro studies were conducted to investigate how the interaction between *C. albicans* and *E. faecalis* impacts the formation of dual-species compared to monomicrobial biofilms grown on polystyrene surfaces. Biomass and cell viability were assessed through colorimetric assays and plate counts, respectively. Virulence gene expression levels were measured by RT-qPCR. To determine whether interactions were mediated by secreted metabolites, biofilms were grown in the presence of heat sterilized or untreated supernatants collected from monomicrobial cultures. The effect of *E. faecalis* supernatants on *C. albicans* hyphal development was evaluated by optical microscopy.

Results

Co-cultures of *C. albicans* and *E. faecalis* produced total biofilm biomass comparable to that of single-species cultures. While *C. albicans* growth remained stable, *E. faecalis* showed

increased colony counts in mixed biofilms. Gene expression analysis revealed, in dual-species biofilm, downregulation of biofilm and virulence genes in *C. albicans* and upregulation in *E. faecalis*. Supernatants from both microorganisms inhibited biofilm formation by the other species even after heat treatment, with *E. faecalis* supernatants also inhibiting *C. albicans* hyphal growth.

Discussion and Conclusions

This study highlights a complex interplay between *C. albicans* and *E. faecalis*, with the latter exerting a predominant antagonistic effect on fungal biofilm formation and virulence factor expression. Conversely, *C. albicans* appears to enhance bacterial viability and virulence gene expression. The activity of heat-stable, cell-free supernatants suggests the involvement of unidentified extracellular metabolites beyond the previously described EntV peptide. Further research is needed to clarify the mechanisms underlying these interactions and to identify key components, potentially leading to the discovery of new therapeutic targets or molecules for the treatment of polymicrobial infections.

281 - Comparative analysis of ultracentrifugation and size-exclusion chromatography methods for the isolation of Outer Membrane Vesicles (OMVs) produced by two unrelated pathogenic Escherichia coli strains

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Comparative analysis of ultracentrifugation and size-exclusion chromatography methods for the isolation of Outer Membrane Vesicles (OMVs) produced by two unrelated pathogenic *Escherichia coli* strains

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Introduction

Outer Membrane Vesicles (OMVs) are proteolipid structures blebbing from bacterial outer membrane, contributing to the release of toxins, antimicrobial resistance factors and molecules involved in the infection process, as demonstrated for Uropathogenic (UPEC) and Shiga toxin-producing *Escherichia coli* (STEC), the primary cause of urinary and gastrointestinal infections in humans, respectively. Conventional techniques, such as ultracentrifugation (UC) and size-exclusion chromatography (SEC), are widely used to isolate OMVs. However, each method may alter the characteristics of the isolated OMVs, impacting their morphology and causing OMV aggregation and unwanted cell debris. Thus, achieving suitable yields and purity of OMVs for research and clinical applications remains challenging. We compared preparations of OMVs produced by an UPEC LC2 strain, from a pyelonephritis case, and a STEC O80:H2 strain, from a milk sample, in terms of morphology, size and concentration, obtained using differential UC (dUC) and SEC, to optimize OMV recovery for downstream studies.

Materials and Methods

For both strains, OMVs were purified *via* dUC using two sequential steps at 100k and 200k x *g* of 2h each, obtaining two OMV-enriched fractions (F1 and F2), and *via* SEC, from the same overnight cultures, in triplicate. The OMVs collected were analyzed by Electron Microscopy (EM) and Nanoparticle Tracking Analysis (NTA) to define their morphology, size and concentration.

Results

EM observation of dUC fractions revealed that, in both strains, F1 contained a high abundance of cellular debris and contaminants, including pili and flagella alongside OMVs. In contrast, F2 of both strains showed decreased levels of debris and contaminants. Thus, F2 was selected for further analyses. As for STEC strain, NTA showed an average concentration of 2,2e+11 particles/ml for F2 preparation and of 9,8e+10 particles/ml for SEC-isolated OMVs, with an average diameter-distribution of 142 nm and 100 nm, respectively. As for UPEC strain, NTA showed an average concentration of 4,6e+10 particles/ml for F2 preparation and of 6,1e+10 particles/ml for SEC-isolated OMVs, with an average diameter-distribution of 149 nm and 159 nm, respectively. In general, F2 from dUC resulted in quite homogeneous particles preparations comparable to SEC-isolated preparations.

Discussion and Conclusions

Our data validated the efficacy of sequential centrifugation in obtaining fractions with an acceptable depletion of unwanted non-OMV materials (F2), which could interfere with downstream investigations. Similarly, SEC method allows the isolation of homogenous and measurable OMV-enriched preparations, comparable to dUC fractions obtained at 200k x *g*. These results may help in selecting the most suitable method for OMV recovery for cellular and molecular applications.

283 - Air-Liquid Interface respiratory model for infection studies with Streptococcus pneumoniae

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Air-Liquid Interface respiratory model for infection studies with *Streptococcus pneumoniae*

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Introduction. *Streptococcus pneumoniae* is a major human pathogen responsible for both local and invasive diseases, including sinusitis, otitis media, pneumonia, sepsis, and meningitis. Nasopharyngeal carriage of *S. pneumoniae* is considered as a reservoir for transmission and a precursor of pneumococcal disease. Pneumococcal carriage varies with age and reaches its highest levels (20-50%) in children under 5 years of age. Air-Liquid Interface (ALI) models represent an innovative and effective tool for investigating the interactions between the pneumococcus and the human respiratory tract.

Materials and Methods. The ALI model was set up starting from human primary nasal epithelial cells. Cells were initially expanded in flasks and then transferred to collagen-coated transwell inserts. Initially maintained in liquid-liquid culture conditions for 2-4 days, the medium was then added only to the basolateral compartment. After 21 days, the ALI model was assessed by immunofluorescence for the presence of specific cell markers (i.e., Muc5Ac, alfa-tubulin, P63, F-actin, ZO-1 and CC10), scanning electron microscopy (SEM) and measurement of transepithelial electrical resistance (TEER). Time-course infection experiments were performed using a GFP-producing *S. pneumoniae* D39 strain (type 2) under two different experimental conditions (continuous and transient exposure) at two different MOIs of infection. At the end of infection, epithelial integrity was assessed, and both bacterial load and distribution were evaluated by viable counts, immunofluorescence and TEER measurements.

Results. Model development was confirmed by assessing formation of tight-junctions and presence of the main cell populations typical of the human respiratory epithelium. Post-infection analyses revealed that MOI 50:1 caused a rapid epithelial damage, while MOI 10:1 preserved barrier integrity. Under continuous exposure condition, bacteria were predominantly extracellular, with levels of tightly adherent and intracellular bacteria decreasing over time. Under transient exposure condition at MOI 10:1, the number of intracellular bacteria showed a modest increase over time, suggesting enhanced bacterial entry into epithelial cells.

Discussion and Conclusions. Most bacteria (>99%) were found extracellularly entrapped in the mucus, while less than 1% were observed inside respiratory cells, under all tested conditions. These findings suggest that the present ALI respiratory model may represent a valuable tool for studying nasopharyngeal carriage by *S. pneumoniae*.

295 - Overcoming the barriers of Gram-positive bacteria: isolation of BEVs from Staphylococcus aureus and Limosilactobacillus fermentum, compared to Gram-negative protocols

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Overcoming the barriers of Gram-positive bacteria: isolation of BEVs from *Staphylococcus aureus* and *Limosilactobacillus fermentum*, compared to Gram-negative protocols

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Introduction: Bacterial extracellular vesicles (BEVs) represent a crucial means of intercellular communication and carriers of bioactive molecules. Isolation of BEVs from Gram-negative ($Gram^-$) bacteria is well established due to the relative fragility of their outer membrane. Conversely, isolation from Gram-positive ($Gram^+$) bacteria is more challenging and less standardized because of the thick and rigid peptidoglycan cell wall. This study proposes an experimental model to isolate BEVs from two $Gram^+$ strains: *Staphylococcus aureus* and *Limosilactobacillus fermentum* isolated from human breast milk.
Materials and Methods: $Gram^+$ bacterial strains will be used as models for BEV isolation. Samples will undergo cell removal followed by an ultracentrifugation protocol. Isolated vesicles will be characterized in terms of size, concentration, and protein content using standard biochemical and analytical techniques.
Results: Ultracentrifugation is expected to effectively isolate BEVs from $Gram^+$ strains, with vesicle size distributions consistent with those previously reported for BEVs. Proteomic analyses will provide a preliminary profile of vesicle content, highlighting potential differences between the strains used and confirming the efficacy of the applied protocol.

Discussion and Conclusions: This study demonstrates that ultracentrifugation can be successfully adapted for Gram$^+$ bacteria, partially overcoming the structural limitations compared to protocols standardized for Gram$^-$. Differences observed between *S. aureus* and *L. fermentum* indicate species-specific variability in vesicle production and composition. The proposed protocol provides a solid foundation for future functional studies of Gram$^+$ BEVs in biomedical and biotechnological fields.

299 - Transcriptomic profiling of Staphylococcus aureus: an in silico approach to explore expression dynamics

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Transcriptomic profiling of *Staphylococcus aureus*: an *in silico* approach to explore expression dynamics

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Introduction. Transcriptomics allows for a systematic and wide-ranging gene expression analysis, offering valuable insights into the molecular mechanisms underlying biological processes in microorganisms. Technologies such as RNA-seq and microarrays generate large amounts of data, which are now widely available through public repositories such as NCBI's Gene Expression Omnibus (GEO). In this context, *Staphylococcus aureus*, a Gram-positive pathogen of clinical relevance, constitutes an ideal model to explore mechanisms of gene adaptation and regulation through *in silico* transcriptomic approaches.

Materials and Methods. Public datasets of *S. aureus* from the GEO database were selected from experiments conducted under distinct experimental conditions. Expression matrices with raw counts were analysed in the Python environment. The workflow included quality control, normalisation, and differential expression analysis (adjusted p-value/FDR < 0.05). Graphical representations (PCA, volcano plot, heatmap) were realised with the seaborn and matplotlib packages. Functional enrichment of differentially expressed genes was performed using online tools to identify GO categories and associated KEGG pathways.

Results. The analysis revealed distinct transcriptional patterns between the experimental conditions. PCA analysis showed a clear separation between samples, indicative of consistent changes in gene expression. Functional enrichment revealed the involvement of genes in metabolic processes, stress response, and membrane functions.

Discussion and Conclusions. This study demonstrates the efficacy of using public transcriptomic datasets to study microbial gene expression. The analysis of GEO data with bioinformatics tools offers a reproducible, accessible, and useful approach to generate new hypotheses in clinical microbiology.

300 - Use of Galleria mellonella as a Preclinical In Vivo Model to Evaluate the Pathogenicity of Intestinal Bacteria and the Protective Role of Human Milk-Derived Lactic Acid Bacteria

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Use of *Galleria mellonella* as a Preclinical *In Vivo* Model to Evaluate the Pathogenicity of Intestinal Bacteria and the Protective Role of Human Milk-Derived Lactic Acid Bacteria

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Introduction: *Galleria mellonella larvae* are a versatile and ethically favorable *in vivo* preclinical model for studying host–pathogen interactions and screening antimicrobial or probiotic compounds. Their innate immune system shares structural and functional similarities with mammals, making them a suitable tool for modeling microbial infections, particularly in the gastrointestinal context. In this study, we used *G. mellonella* to evaluate the virulence of intestinal bacterial species and to test the *in vivo* protective effect of Lactic Acid Bacteria (LAB) strains isolated from human breast milk.
Materials and methods: *Larvae* were infected using a refined oral gavage technique. Pathogenic bacterial suspensions were prepared in phosphate-buffered saline and administered defined inocula directly into the larval foregut. Survival was recorded at 48 hours post-infection. Four different LAB strains isolated from human milk were pre-administered via

oral gavage before pathogenic challenge. Control groups included uninfected *larvae* and *larvae* receiving only LAB.

Results: All pathogens induced significant mortality, confirming their virulence in *G. mellonella*. Pre-treatment with LAB strains resulted in a statistically significant increase in survival at 48 hours post-infection. The protective effect varied depending on the combination of LAB strain and pathogen, suggesting strain-specific antagonistic activity.

Discussion and Conclusions: These findings demonstrate the relevance of *G. mellonella* as a reliable, reproducible, and cost-effective preclinical model for investigating the pathogenicity of enteric bacteria and evaluating probiotic interventions. The observed protective role of human milk-derived LAB strains supports their potential application in preventing intestinal infections. This model may serve as a valuable high-throughput platform to screen probiotic candidates before advancing to mammalian studies, reducing animal use in early experimental phases.

302 - The GtgA effector explains the difference in NF-κB-mediated inflammatory response induced by attenuated Salmonella Derby compared to virulent Salmonella Typhimurium

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The GtgA effector explains the difference in NF-κB-mediated inflammatory response induced by attenuated *Salmonella* Derby compared to virulent *Salmonella* Typhimurium

Introduction

Salmonella enterica serovars Derby (*S*. Derby) and Typhimurium (*S*. Typhimurium) are the second and third most frequently isolated serovars from pigs and pork products in the European Union, respectively. Despite similar levels of human exposure to these two serovars, the number of salmonellosis cases caused by *S*. Derby is approximately ten times lower than those caused by *S*. Typhimurium. The underlying reason for the lower virulence of *S*. Derby remains unknown. The invasion of the intestinal epithelial cells (IECs) is a central step of *Salmonella* infection cycle. Therefore, we tested if the attenuation of *S*. Derby was due to its limited ability to invade IECs. Invasion assays performed on one *S*. Derby strain and one *S*. Typhimurium strain showed that strains infect a comparable number of intestinal epithelial cells (IECs). We thus hypothesized that the attenuation of *S*. Derby was related to a different inflammatory response induced in infected IECs compared to *S*. Typhimurium.

Materials and Methods

We conducted RNA sequencing (RNA-seq) to analyze the transcriptomic profiles of IECs infected by *S*. Derby and *S*. Typhimurium. Subsequently, we investigated the genetic basis for the observed differences in modulation of IECs NF-κB proinflammatory pathway. We screened 4,495 *S*. Derby genomes available in the Enterobase database for the presence of the genes *pipA*, *gtgA*, and *gogA*, which are known to encode effectors in *S*. Typhimurium that inhibit NF-κB signaling. To assess the impact of the absence of *gtgA* in *S*. Derby, the strain belonging to this serovar was complemented with a plasmid encoding *gtgA*. Quantitative PCR was then used to measure the expression levels of TNF, CXCL8, and ICAM1—genes belonging to the NF-κB pathway—in IECs infected with the wildtype and complemented *S*. Derby strains, as well as with *S*. Typhimurium.

Results

RNA-seq analysis revealed that *S*. Derby induces higher expression of genes belonging to NF-κB proinflammatory pathway in infected IECs compared to *S*. Typhimurium. Genomic analysis of *S*. Derby showed that while *pipA* was present in all examined genomes, *gtgA* and *gogA* were absent. Following transformation of *S*. Derby with a plasmid encoding *gtgA*, expression of TNF, CXCL8, and ICAM1 in infected IECs decreased to levels similar to those observed in S. Typhimurium infections.

Discussion and Conclusion

These findings indicate that the higher activation of the NF-κB proinflammatory pathway by *S*. Derby is due to the absence of the *gtgA* gene, which in *S*. Typhimurium contributes to dampening host inflammatory responses. Investigations are ongoing to understand how this affects the evolution of *S*. Derby infection.

305 - Potential antimicrobial peptides to fight against orthopaedic infections

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Potential antimicrobial peptides to fight against orthopaedic infections

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Introduction
Periprosthetic infection is a serious complication in orthopaedic surgery associated with the use of implants. The challenge in the management of implant-associated infections is the constant increase of multidrug-resistant bacteria and the persistence of microorganisms on the implant surface in the form of biofilm difficult to eradicate due to a higher antibiotic-tolerance. Current preventive and therapeutic strategies are increasingly focusing on the use of new bioactive molecules that are alternative to antibiotics, such as antimicrobial peptides (AMPs). The aim of this study was to characterize the activity of selected AMPs as potential candidates for the development of novel anti-infective coatings for resurfacing orthopaedic implants.
Materials and Methods
Bacterial inoculum (1×10^6 CFU/mL) was prepared from overnight cultures and exposed to 2-fold serial dilutions of the test AMP. MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration) and MBIC (Minimum Biofilm Inhibitory Concentration) values of each AMP were evaluated in both undiluted and diluted Mueller-Hinton Broth II (MHB II) to test the potential interference of chemical components of MHB II medium. MIC was determined by two distinct methods: optical density measurement and bioluminescence reading. To fully explore their true potential, cytocompatibility of AMPs was also assessed. Experiments were carried out in human osteoblast-like MG63 cells, both in the presence and absence of bovine serum, using the ATP bioluminescence assay.
Results
All AMPs showed broad-spectrum antibacterial activity with MIC values ranging from 0.49 to 31.25 µg/mL in diluted medium and with significant bactericidal properties. Moreover, all test peptides in diluted medium were highly effective even in preventing biofilm formation with MBIC values in the range of 0.24-31.25 µg/ml. Conversely, in undiluted medium, the antibacterial activity of the AMPs underwent pronounced variations, especially in the case of Dadapin-1, which lost its activity. Finally, all AMPs demonstrated good cytocompatibility, exhibiting minimal to no cytotoxicity in medium containing serum, with IC50 values exceeding 100 µg/ml.
Conclusions
Our results demonstrate that all three investigated peptides possess significant broad-spectrum antibacterial properties against both Gram-negative and Gram-positive bacterial

species typically involved in orthopaedic infections, without significant cytotoxicity. Overall, our findings highlight the potential of these AMPs for the future development of anti-infective materials.

306 - Analysis of ESKAPE Pathogens involved in Bloodstream Infection at the University Hospital of Palermo

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"Analysis of ESKAPE Pathogens involved in Bloodstream Infection at the University Hospital of Palermo"

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Introduction

Epidemiological surveillance and prompt alerts are essential for effectively managing bloodstream infections in the hospital setting. Patterns of pathogen spread can provides valuable support for clinical monitoring and the development of targeted prevention and control strategies for HAI.

Materials and Methods

We conducted a study on ESKAPE pathogens, including Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii*) and Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium*), that are frequently involved in nosocomial infections. Blood cultures were collected from hospitalised patients between June 2024 and February 2025. MALDI-TOF was used to identify the bacterial species.

Results

A total of 13,774 blood samples were analysed by the Microbiology and Virology Unit of the University Hospital of Palermo. Of these, 11,556 were negative, while 2,218 showed bacterial growth as detected by the BD BACTEC™ Blood Culture System. Positive cultures were subcultured in an appropriate growth medium and incubated at 37°C for 24-48 hours. MALDI-TOF mass spectrometry identified ESKAPE pathogens in 17% (n = 379) of the positive samples. Specifically, *K. pneumoniae* (n = 98) was isolated in 26% of cases, *E. coli* (n = 74) in 19%, *S. aureus* (n = 69) in 18%, *A. baumannii* (n = 57) in 15%, *P. aeruginosa* (n = 34) in 9%, E. *faecalis* (n = 28) in 7%, and *E. faecium* (n = 21) in 6%.

Discussion and Conclusions

Epidemiological surveillance of bacteria responsible for bacteraemia and sepsis enabled the definition of the microbiological profile of circulating pathogens during the study period. Timely

and affordable identification of ESKAPE pathogens allows to optimise targeted antibiotic therapy, and support prevention efforts against the spread of resistant strains.

314 - Cystic fibrosis-like physicochemical conditions impact the production of the outer membrane vesicles by Pseudomonas aeruginosa.

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Cystic fibrosis-like physicochemical conditions impact the production of the outer membrane vesicles by *Pseudomonas aeruginosa*.

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Introduction: In people with cystic fibrosis (CF), *Pseudomonas aeruginosa* causes chronic biofilm-based infection, leading to a progressive functional decline. In CF lungs, *P. aeruginosa* secretes Outer Membrane Vesicles (OMVs) of 50-300 nm in diameter, involved in cell-to-cell communication, biofilm formation/stabilization, virulence and pathogenesis, immune response/modulation, and colonization as well as in antimicrobial resistance. This study aimed to test the hypothesis that "CF-like" conditions can impact OMVs formation and biological and physicochemical traits such as size, zeta-potential, and vesicle population morphology. **Materials and Methods**: Planktonic and biofilm cells of *P. aeruginosa* MG3871, causing chronic infection, with high potential for biofilm formation, were grown at 37°C under CF-like (artificial sputum medium, 5% CO_2, pH 6.8) and enriched (Tryptone Soy broth, aerobiosis, pH 7.3) conditions. The OMVs were filtered by a 0.22 µm filter and isolated by ultracentrifugation. Physicochemical and biological analysis were conducted to characterize vesicles from planktonic and sessile cells via Nanoparticle Tracking Analysis (NTA), Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). **Results**: NTA showed that planktonic and biofilm cells produced more OMVs under CF-like conditions than in an enriched setting (planktonic, mean±SD: $1.2\pm0.1x10^{13}$ *vs*. $2.3\pm0.5x10^{10}$ particles/ml, respectively, $p<0.0001$; biofilm: $1.8\pm0.8x10^{12}$ *vs*. $1.1\pm0.6x10^{11}$ particles/ml, respectively, $p<0.05$). The mean±SD diameter was: 247.6±11.6 *vs*. 123.4±7.4 nm ($p<0.0001$), for planktonic and biofilm cells in "enriched" medium, respectively; 150.6±10 *vs*. 97.2±8.5 nm ($p<0.0001$), for planktonic and biofilm cells in "CF-like" conditions, respectively. Compared with the "enriched" condition, *P. aeruginosa* planktonic and biofilm cells produced smaller OMVs under CF-like conditions. DLS

indicated that OMVs obtained from “enriched” conditions had a broader size distribution and greater heterogeneity than OMVs obtained under “CF-like” conditions. Planktonic OMVs obtained from “enriched” medium showed the greatest polydispersion compared with the other samples. Regarding the Z potential, CF-like conditions promote formation of OMVs with higher negative values (mean±SD: -6.115±0.653 *vs*. -4.015±0.194 mV, respectively, $p<0.05$). TEM analysis confirmed the data obtained via DLS and NTA, showing intact spherical structures ranging from 50 to 250 nm in diameter. **Discussion and Conclusions**: Our findings indicated that considering physicochemical conditions relevant to the site of infection is critical to obtain OMVs as a starting point for developing future OMVs-based therapeutic systems for lung infections in CF patients.

319 - Optimization of an airway-on-a-chip valuable model for pathophysiology studies and drug testing in a CF-like microenvironment.

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Optimization of an airway-on-a-chip valuable model for pathophysiology studies and drug testing in a CF-like microenvironment.

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Introduction: The development of new therapeutics for cystic fibrosis (CF) is limited by the lack of reliable preclinical models that recapitulate human CF lungs' structural and immunological features. In this study, we optimized a human CF Airway Chip to reproduce many of the human CF lung airway's structural, biochemical, and pathophysiological features and study the response to *Pseudomonas aeruginosa* infection. **Materials and Methods:** CF human bronchial airway epithelial cells (HBE) were cultured and differentiated into pseudostratified epithelia under Air Liquid Interface (ALI) conditions on the top side of 0.4 µm pore diameter transwell inserts and in the top channel of the Emulate® S1 Chip. For cultures on chip, HBE were lined by endothelial cells, cultured in the bottom channel, to simulate a blood vessel with a dynamic flow. GFP-*P. aeruginosa* ATCC10145 strain was used to optimize the bacterial exposure duration and the multiplicity of infection (MOI). Acute infection of the *in vitro* airways was achieved by incubating bacteria for 1 hour in the epithelial side of the 3D cultures, and subsequent restoration of the ALI to allow bacterial growth within the mucus. Bacterial counts and distribution in the 3D cultures were evaluated throughout CFU count and confocal microscopy, respectively. Proinflammatory cytokine/chemokine levels were evaluated, while metabolic assay and Trans-Epithelial Electrical Resistance (TEER) were used to evaluate cytotoxicity and epithelial barrier integrity, respectively. **Results**: The CF Airway Chip effectively mimicked several characteristics of human CF airways, including an abundant mucus layer and a baseline inflammation. *P. aeruginosa* infection showed a reduction in TEER values, but not a significant cytotoxicity, indicating adequate MOI and incubation time. Moreover, infection with *P. aeruginosa* led to the secretion of inflammatory cytokines and evidence of microcolony formation by confocal microscopy. **Discussion and Conclusions**: Our findings will open the way to create personalized preclinical models for mechanistic studies as well as drug testing and discovery that could help to accelerate the delivery of more effective therapeutics to patients.

Project's title:"Optimized in vivo and ex-vivo Models as Platforms for the Preclinical Evaluation of Antimicrobial lead Compounds - OMPEAC". Codice progetto ECS00000017. Funded by the European Union - Next Generation EU. Views and opinions expressed are however those of the

author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them. PNRR MUR M4 C2 Inv. 1.5 CUP Master B63C22000680007 CUP di progetto: D93C22001360007.

339 - In vitro adaptation of the A/Puerto Rico/8/1934 influenza virus (H1N1) to the murine lung adenoma cell line LA-4

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In vitro **adaptation of the A/Puerto Rico/8/1934 influenza virus (H1N1) to the murine lung adenoma cell line LA-4**

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1.Introduction

Influenza A virus is an enveloped virus with eight negative-sense RNA gene segments, causing seasonal epidemics and pandemics of respiratory illness. *In vivo* studies in mouse models are limited and require several animal passages of the virus, since mice are not naturally susceptible to influenza A. In this study, the A/Puerto Rico/8/1934 (H1N1) [PR8] influenza A virus was adapted *in vitro* to mouse lung tissue cells to facilitate subsequent *in vivo* studies on pathogenesis and vaccine formulation.

2. Materials and Methods

PR8 was purchased from the American Type Culture Collection (ATCC), amplified with 3 passages in MDCK cells, followed by 45 serial passages in LA-4 mouse lung adenoma cells. Virus titration was performed with TCID50 assay. Viral passages 5, 15, 25, 35 and 45 were subjected to replication efficiency assays and complete genome sequencing using amplicon sequencing with Nanopore technology. Additionally, growth kinetics were evaluated and compared between the wild-type virus and passage 45.

3. Results.

Replication efficiency analysis showed a statistically significant increase in viral replication after passage 35 in LA-4 cells, when compared to the 5th passage. Genomic analysis revealed a progressive accumulation of single nucleotide polymorphisms (SNPs) across the viral passages, with 8, 13, 14, and 15 SNPs detected at passages 15, 25, 35, and 45, respectively. Mutations were present in all gene segments except for the polymerase basic 2 (PB2) gene. Out of the fifteen SNPs at passage 45, six did not result in predicted amino-acid changes, while the remaining nine resulted in amino acid changes in hemagglutinin (HA), matrix protein (MP), neuraminidase (NA), non-structural protein (NS) and the acidic polymerase (PA). Passage 45 of PR8 showed a marked increase in TCID50 titer compared to wild-type PR8.

4. Discussion and Conclusions

We developed and characterized a A/Puerto Rico/8/1934 (H1N1) influenza virus strain adapted to LA-4 murine cells. This adapted strain could be used in mouse models for studying infections and experimental vaccine protection, eliminating the need for *in vivo* serial passages.

349 - Protein-Based Nanoparticle-Coated Gauzes for Enhanced Antibacterial Wound Care

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Protein-Based Nanoparticle-Coated Gauzes for Enhanced Antibacterial Wound Care

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INTRODUCTION. Chronic wounds pose a significant clinical challenge, often exacerbated by infections from antibiotic-resistant microorganisms. Advanced wound dressings capable of protecting the wound and locally delivering therapeutic agents are gaining interest in regenerative medicine. One promising strategy is the functionalization of medical gauzes with drug-loaded nanoparticles to enable sustained release and enhanced antimicrobial activity. In this study, cotton and non-woven commercial gauzes were coated with protein-based nanoparticles designed to encapsulate and deliver ciprofloxacin. The modified gauzes were evaluated for nanoparticle adhesion, drug release kinetics, and antibacterial efficacy, against Gram positive and Gram negative bacteria implicated in wound infections.

MATERIALS AND METHODS. Cotton and non-woven commercial gauzes (1 cm^2) were immersed in a protein-based nanoparticle suspension (0,3 mg/mL) containing ciprofloxacin and incubated at room temperature for 24 hours on agitation. Then, the concentration of ciprofloxacin remaining in the supernatant was determinated via UV-Vis spectroscopy at 275 nm. The excess liquid was removed and the gauzes were air-dried. For drug release analysis, the nanoparticle-coated gauzes were incubated in 1 mL of simulated human sweat at 37 °C and the ciprofloxacin release was quantified at different time points. Antibacterial efficacy was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, choose as representative bacteria implicated in wound infections, using an agar diffusion assay.

RESULTS. Nanoparticles were successfully deposited onto medical-grade gauzes via a 24-hour soaking process, with deposition efficiencies exceeding 20%. Non-woven gauzes showed higher drug release than cotton ones, due to their structure. Ciprofloxacin release followed a biphasic pattern: a rapid initial phase followed by sustained release. After 24 hours, non-woven and cotton gauzes released ~60% and ~40% of the drug, respectively. Antibacterial tests confirmed effective drug release by nanoparticle-coated gauzes, in particular cotton ones, producing significantly larger inhibition zones than those treated with free drug against both bacteria.

DISCUSSION AND CONCLUSIONS. This study demonstrates the successful functionalization of medical gauzes with protein-based nanoparticles using a simple soaking method. The biphasic drug release results ideal for wound healing and antibacterial tests confirmed that nanoparticle-coated gauzes, in particular cotton ones, offer a promising approach for advanced wound care, combining efficient drug loading, controlled release, and superior antibacterial efficacy. Hence, they could potentially be usable as a novel platform for smart wound care materials with improved therapeutic performance.

350 - A mRNA-based vaccine approach against bacterial diseases: the Streptococcus pneumoniae model.

Marta ACCHIONI

A mRNA-based vaccine approach against bacterial diseases: the *Streptococcus pneumoniae* model.

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Introduction: mRNA vaccine technology has demonstrated an unprecedented translational potential characterized by high efficacy and safety during the COVID-19 pandemic. In addition to SARS-CoV-2, mRNA platforms also showed strong potential as vaccines against other viral pathogens, such as Zika or influenza virus, but applications against bacterial diseases have been sporadically approached so far. Prevention of diseases caused by *Streptococcus pneumoniae* (Spn) relies on 2 available vaccines: a polysaccharide vaccine (PPV) or protein-conjugated polysaccharide vaccines (PCVs), but several limitations call for improving the strategy for vaccinations against Spn diseases. **Materials and Methods**: We encapsulated within liposomes *in vitro*-synthesized mRNA coding for the proline-rich domain of PspA1 (D39) and PspA2 (TIGR4) and mRNA coding for pneumolysin (Ply) toxoid (PlyT). Mice were immunized and sera were tested to measure antigen specific antibodies by ELISA, and Ply neutralization assay. Groups of female CD1 outbred mice were infected with 10^6 CFU/mouse of the D39 strain of Spn non-opsonized or opsonized with specific anti PspA serum. **Results**: Mice immunized twice with mRNA coding for PspA1 showed a notable specific antibody production in three independent experiments. Mice immunized with mRNA coding for PspA2, on the contrary, did not develop a specific antibody response. However, some mice produced high antibody titers when immunized with the home-made recombinant PspA2 protein. Survival assessment (equivalent to the time of reaching moderate signs) showed significant protection conferred by the specific anti-PspA serum obtained by vaccination with mRNA at 8 µg. **Discussion and Conclusions**: Our preliminary results indicate that mRNA vaccine technology can be exploited not only in the prevention of viral diseases, but also in the fight against bacterial diseases, if the mRNA codes for an appropriate protein antigen and represent a proof of concept for a mRNA based vaccination

tailored for diseases caused by extracellular bacteria and a preclinical phase for a possible future trial.

354 - Host-Pathogen interactions between Klebsiella pneumoniae and CaCo-2 cells reveal an invasive ability, potentially relevant to dissemination

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Host-Pathogen interactions between *Klebsiella pneumoniae* and CaCo-2 cells reveal an invasive ability, potentially relevant to dissemination

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Introduction

Klebsiella pneumoniae is a Gram-negative bacterium responsible for a broad spectrum of infections, frequently associated with antimicrobial resistance. Traditionally considered an extracellular pathogen, emerging evidence supports a facultative intracellular lifestyle. This study explores host-pathogen interactions between clinical *K. pneumoniae* isolates and human colorectal adenocarcinoma CaCo2 cells, hypothesizing that in patients with intestinal colonization followed by bloodstream infection, dissemination may involve intracellular mechanisms.

Materials and Methods

Three *K. pneumoniae* strains were analyzed: ATCC 700603 and two clinical isolates from patients with documented intestinal colonization preceding bloodstream infection. Whole genome sequencing (Illumina) was used to characterize their virulome and resistome. CaCo-2 cells, which form a polarized monolayer mimicking the intestinal barrier, were infected at 5×10^5

cells/well using a multiplicity of infection of 100:1 for 2 hours. Adherence, internalization, persistence, and intracellular replication were assessed via colony-forming unit (CFU) counts after treatment with 4 µg/mL colistin, to kill extracellular bacteria, and host cell lysis. Expression of 11 virulence-associated genes was analyzed by RT-qPCR: *galF, wzi, manC, rcsA, rcsB, rmpA, rmpA2* (capsule); *fimH, mrkA* (fimbriae types 1 and 3); *hcp, vgrG* (type VI secretion system). Expression profiles were compared among pure cultures, extracellular bacteria, and bacteria adhered to or internalized in CaCo2 cells.

Results

All tested strains exhibited not only adhesion and internalization but also intracellular persistence and replication, reaching up to ~10^4 CFU/mL intracellularly, after extracellular killing and host cell lysis at 2, 4, and 6 hours after colistin treatment. Preliminary findings, still ongoing, suggest that, compared to pure culture conditions, capsular genes were underexpressed in cell-associated bacteria, while fimbrial and secretion system genes were overexpressed in extracellular, adhered, and internalized populations.

Discussion and Conclusions

These initial results suggest that *K. pneumoniae* is capable of adopting an intracellular lifestyle in intestinal epithelial cells, potentially acting as a reservoir and contributing to systemic dissemination, immune evasion, and treatment failure. The observed gene expression shifts point to adaptive mechanisms triggered by host interaction. Further analyses, including flow cytometry, confocal microscopy, and infections with gene knockout strains, are ongoing to corroborate these findings. This study challenges the conventional view of *K. pneumoniae* as strictly extracellular pathogen, highlighting novel pathogenic pathways and potential therapeutic targets.

02 2. Infezioni fungine e parassitarie

47 - Targeting Leishmania through chalcone derivatives: a comprehensive in vitro and in silico study

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Targeting *Leishmania* through chalcone derivatives: a comprehensive *in vitro* and *in silico* study

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Introduction

Current treatments of leishmaniasis are limited by toxicity, drug resistance, high costs, and difficult administration. Thus, safer and more effective therapies targeting parasite-specific pathways are urgently needed to fight this neglected tropical disease. In this context, newly synthetized chalcones, naturally derived flavonoid precursors, were evaluated *in vitro* and *in silico* for their antileishmanial activity.

Materials and Methods

Chalcones were synthesized via Claisen–Schmidt aldol condensation, followed by functionalization of the scaffold rings. Their antiparasitic activity was evaluated *in vitro* against *Leishmania infantum* promastigotes, and IC_{50} - IC_{90} values (the concentrations required to inhibit 50% or 90% of parasite growth) were determined after 72 hours using the Alamar Blue assay. Toxicity was assessed on human U937 cells, and the selectivity index (SI) was calculated. For the most active compounds, inhibitory effects were also evaluated on intracellular amastigotes, through microscopic analysis, and on promastigotes of other *Leishmania* species (*L. donovani, L. major, L. tropica*). Finally, *in silico* modeling of

trypanothione reductase (TR) and molecular docking studies were performed to predict compound–target interactions.

Results

Twenty-four novel chalcone compounds were synthesized and their activity was evaluated against promastigotes of *Leishmania infantum*; four compounds – C1, C2, C5, and C12 – showed potent inhibitory effects on promastigotes of four *Leishmania* species, with IC_{50} values in the low micromolar range. Lead compounds also inhibited the intracellular amastigote form, confirming their efficacy in a host-cell environment. The SI values indicated that these lead compounds were selectively toxic to the parasite and not to human cells. Docking studies revealed that C1 and C2 bound strongly to the FAD-binding site of TR (-11.5 and -10.8 kcal/mol), a crucial region for enzymatic activity. Weaker but still relevant interactions were observed at the NADPH, ubiquinone, and trypanothione sites, suggesting possible multitarget effects at higher doses. When tested on other parasite flavoproteins like TR, all leads showed binding activity, but C2 displayed highest selectivity for TR, helping reduce off-target effects.

Discussion and Conclusion

We identified promising chalcone derivatives with consistent in vitro efficacy against different Leishmania species and favorable selectivity. The strong binding to TR, particularly at the FAD site, supports TR as a viable drug target. These findings encourage further optimization of chalcones as anti-leishmanial agents, leveraging their selective interference with parasite-specific metabolic pathways.

68 - Isavuconazole and Amphotericin B Synergic Activity:

in vitro evaluation on Aspergillus spp. clinical isolates

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Isavuconazole and Amphotericin B Synergic Activity:

in vitro **evaluation on** ***Aspergillus*** **spp. clinical isolates**

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Introduction

Invasive pulmonary aspergillosis (IPA) is a critical respiratory infection caused by *Aspergillus* spp. Immunocompromised or intensive care unit (ICU) patients represent a prominent target, especially due to neutropenia, mechanical ventilation, and viral respiratory syndromes. Triazoles and liposomal amphotericin B are fundamental therapeutical options, but potential resistance and/or toxicity episodes led to antifungal synergism hypotheses. Herein, we propose an *in vitro* investigation of the synergic isavuconazole and amphotericin B activity on *Aspergillus* spp. isolated from IPA patients.

Materials and Methods

We included *Aspergillus* spp. strains from respiratory samples of University Hospital Policlinico (Catania) patients' during a three years (2022-2024) interval, testing isavuconazole/amphotericin B susceptibility (MIC Strip method), together with an EUCAST broth microdilution confirmation. MIC values were analyzed according to EUCAST clinical breakpoints or epidemiological cut-offs (E-COFF). Isavuconazole and amphotericin B strips were arranged to investigate synergy, calculating the FIC index. The authors evaluated a statistical significance between identified species and synergy test result.

Results

We collected 57 *Aspergillus* spp. strains from respiratory samples of intensive care (31), haematology (13), pneumology (5), and infectious diseases (8) units' patients. We identified *A. fumigatus* (22), *A. flavus* (14), *A. terreus* (11), and *A. niger* (10). All the strains revealed isavuconazole susceptibility. *A. fumigatus* showed a complete amphotericin B susceptibility. One *A. niger* reported resistance and 3 *A. flavus* (3) had MIC higher than the E-COFF. The MIC Strip method MIC values were comparable to the EUCAST broth dilution ones. *A. fumigatus* was the only species reporting synergy (13.6%), achieving the highest additivity (36.4%) and the lowest antagonism (9.1%) rates. *A. terreus* showed the highest indifference rate (54.5%), while *A. niger* reported the highest antagonism percentage (70%) and the lowest additivity rate (10.0%). The statistical significance about synergy tests only emerged for *A. fumigatus* ($p<0.05$).

Discussion and Conclusions

Our study demonstrated a species-specific attitude when antifungal combinations act against *Aspergillus* spp. isolates, highlighting the importance of a correct fungal species identification. Isavuconazole and amphotericin B expressed *in vitro* synergy or additivity against *A. fumigatus*, reporting a statistical significance. Otherwise, *A. niger* and *A. flavus* showed a high antagonism rate, along with the *A. terreus* indifference percentage. Our data enhanced the importance of further investigation on antifungal synergic activity and molds isolates, including more strains and other antifungal drugs.

70 - In vitro activities of two novel peptides against clinical isolates of Candida species including fluconazole-resistant strains

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In vitro **activities of two novel peptides against clinical isolates of *Candida* species including fluconazole-resistant strains**

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1. Introduction. Fungal infections caused by *Candida* species represent an increasing clinical threat, particularly due to the emergence of strains resistant to conventional antifungal agents. Identifying new therapeutic strategies is therefore a priority. In this study, we evaluated the antifungal efficacy of two antimicrobial lipopeptides Nel and WR, as potential alternatives to conventional treatments. 2. Materials and methods. Eight isolates of *Candida* species including two isolates each of *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* were tested. Fluconazole-resistant strains were included in the analysis. Antifungal activities were

assessed by broth microdilution tests following EUCAST guidelines. Additionally, fungal growth was monitored over 24 hours by measuring opical density at 450 nm and counting colony-forming units (CFU/ml) at concentration of 0,5xMIC, 1xMIC and 2xMIC. 3. Results. Nel and WR peptides exhibited effective antifungal activity against all tested isolates, including fluconazole- resistant strains (MIC> 128 µg/ml) with MIC values of 4 µg/ml. Growth analysis revealed that at 1xMIC and 2xMIC, peptides significantly reduced cell density (CFU/ml) compared to the untreated control. Serial dilution plate assays confirmed a progressive decrease in viable colonies after 6 hours after treatment, with a more pronunced effect at 12 and 24 hours. 4. Discussion and Conclusions. Nel and WR peptides emerge as promising therapeutic alternatives to conventional antifungal agents, demonstrating effective in vitro activity even against fluconazole-resistant *Candida* strains. Further preclinical studies are needed to validate their clinical applicability.

86 - Mucosomes as Potential Inhibitors of Candida albicans Virulence Factors: Impact on Biofilm and Pseudohyphal Formation

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Mucosomes as Potential Inhibitors of *Candida albicans* Virulence Factors: Impact on Biofilm and Pseudohyphal Formation

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INTRODUCTION. Mucosal candidiasis, including oral and vulvovaginal infections, represents a significant clinical challenge, especially in immunocompromised individuals. One of the major obstacles in treating these infections lies in the ability of *Candida albicans* to form biofilms that confer resistance to antifungal agents and host immune responses. Additionally, its morphological plasticity, particularly the transition from yeast to filamentous forms like pseudohyphae, is a key virulence factor that enhances tissue invasion and adhesion to epithelial surfaces. These features contribute to the persistence and recurrence of infections, making eradication difficult. Understanding the virulence mechanisms of *C. albicans* is crucial for the development of more effective and targeted therapeutic strategies. In this context, we are currently investigating the potential of mucosomes as inhibitors of pseudohyphal development and biofilm formation. Mucosomes—mucus-derived vesicular structures—may offer a novel and physiologically relevant approach to interfering with key virulence traits of *C. albicans*, potentially reducing fungal pathogenicity without relying solely on conventional antifungal drugs.

MATERIALS AND METHODS. For biofilm analysis, two strains of *C. albicans*—a azole-susceptible and an azole-resistant isolate—were inoculated into 96-well microtiter plates. RPMI 1640 medium buffered with MOPS and supplemented with glucose (RPMI-MOPS-glu) was

used as the culture medium. Mucosomes (NPs), prepared with mucine from bovine submaxillary gland (BSM), were added at time zero in selected wells to assess their effect on biofilm formation (pre-formation inhibition), while in another set of wells, NPs were added 24 hours post-inoculation to evaluate their impact on mature biofilms (post-formation inhibition). Plates were incubated at 37 °C, and biofilm development was evaluated 24 hours after NPs addition. Biofilm biomass was quantified by measuring the optical density at 595 nm (OD_{595}) using a VICTOR$^{3™}$ microplate reader (PerkinElmer).
To evaluate pseudohyphal formation, a 96-well microtiter plate assay was set up including negative controls (containing *yeasts* and fetal bovine serum), a positive control (containing yeasts, serum, and an azole antifungal), and test wells containing yeasts, serum, and mucosomes at various concentrations. The plates were incubated at 37 °C, and pseudohyphal development was assessed by optical microscopy at 100× magnification after 24, 48, and 72 hours.

RESULTS. The results suggest that mucosomes may contribute to the reduction of key virulence factors in *C. albicans*. In particular, a decrease in biofilm biomass was observed, especially in the drug-resistant strain, indicating a potential selective effectiveness of NPs under conditions of antifungal resistance. Additionally, a reduction in pseudohyphal formation was noted across different time points and concentrations.

DISCUSSION AND CONCLUSIONS. These findings support the hypothesis that mucosomes can interfere with critical morphological and structural adaptations of *C. albicans*, highlighting their potential as a novel strategy for attenuating fungal virulence and complementing existing antifungal therapies.

118 - Comparison of Molecular Methods for Blood-Based Detection of Leishmania infantum: A Retrospective Analysis of Diagnostic Accuracy

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Comparison of Molecular Methods for Blood-Based Detection of *Leishmania infantum*: A Retrospective Analysis of Diagnostic Accuracy

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Introduction

Visceral leishmaniasis (VL) is a life-threatening and re-emerging parasitic disease caused by *Leishmania infantum* in the Mediterranean basin. Molecular diagnostics, particularly in-house PCR assays, have become increasingly used for the detection of VL. In recent years, the development of commercially available PCR-based kits has aimed to improve VL identification and enhance the standardization.

Materials and Methods

In this retrospective study, we evaluated the diagnostic performance of two real-time PCR-based assays routinely employed for the detection of visceral leishmaniasis (VL) at the Regional Reference Laboratory for Leishmaniasis (RRL) in Emilia-Romagna, northern Italy. The first assay, a commercially available platform, employs a fully automated workflow for both DNA extraction and amplification, targeting the 16S ribosomal RNA gene (rDNA) of *Leishmania*. The second assay is an in-house developed method implemented at the RRL, follows a semi-automated protocol that targets two distinct *Leishmania* genomic regions: the rDNA and the

kinetoplast DNA minicircle (kDNA). A total of 90 peripheral blood samples from patients presenting with clinical suspicion of VL at the University Hospital of Bologna were included. Of these, 33 were confirmed as VL cases, while 57 tested negative for the disease.

Results

We observed higher sensitivity (100%) for the amplification of the kDNA target using the in-house method compared to the amplification of rDNA using both the in-house (97%) and commercial PCR (94%). Conversely, kDNA showed slightly lower specificity (96%) on clinical samples compared with rDNA (100%). Both methods exhibited excellent concordance in VL identification (Cohen's $k > 0.8$). The superior sensitivity of the kDNA target was validated by comparing the Ct values obtained from clinical samples amplification across the three real-time PCR studied (Pairwise Wilcoxon test; p-value < 0.0001).

Discussion and Conclusions

In conclusion, our findings demonstrated strong diagnostic performance for both molecular approaches, with the kDNA target displaying superior sensitivity compared to rDNA. This enhanced sensitivity is particularly crucial in cases of low parasitemia, where diagnostic inaccuracies may lead to severe clinical consequences. Therefore, we emphasize the need for the development and implementation of automated, commercially available diagnostic platforms targeting kDNA to improve the accuracy and reliability of VL diagnosis.

178 - Chromosome-level genome assembly of Magnusiomyces clavatus from a hospital cluster in northern Italy, 2024

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Chromosome-level genome assembly of Magnusiomyces clavatus from a hospital cluster in northern Italy, 2024

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Introduction

Magnusiomyces clavatus is an emerging opportunistic fungus closely related to and often misidentified with *M. capitatus*. These fungi are intrinsically resistant to echinocandins and several reports demonstrated the ability of *M. clavatus* to elicit the onset of severe opportunistic diseases in immunocompromised patients with a mortality up to 80% in patients with hematological diseases or AIDS. Genomic data for *M. clavatus* is limited as well, with a reference with a scaffold-level genome assembly including 339 scaffolds and no assembled chromosomes. In this study, we provide the first attempt to obtain the complete genome sequence resolved at chromosomal level.

Materials and Methods

Three clinical fungal isolates were identified as *M. clavatus* by MALDI-TOF MS, and antifungal susceptibility tests were carried out. DNA was extracted with Zymo Research Quick-DNA™

Fungal/Bacterial Miniprep Kit (Irvine, CA), followed by whole genome sequencing (WGS) using Illumina MiSeqDx and Nanopore MinION Mk1B. Data processing included trimming with Trimmomatic and Porechop, *de novo* genome assembly using Flye, assembly quality control with Merqury, Quast and BUSCO, and annotation with Funannotate and InterProScan. Further improvements were obtained using *M. capitatus* reference genome as scaffold for RagTag, followed by a single Pilon iteration.

Results

The final assembly of *M. clavatus* resulted in a genome of 17.6 Mb, organized into four chromosomes and one mitochondrial genome. Phylogenetic analysis revealed that the three isolates belonged to a newly identified clade (D), distinct from the previously described clades (A-C). Antifungal susceptibility tests confirmed intrinsic resistance to echinocandins: mutations in the 1,3-beta-glucan synthase gene, responsible for echinocandin resistance were detected, similar to those in resistant *M. capitatus* strains. Additionally, an analogue of the azole-targeted *cyp51* gene was identified, possibly contributing to fluconazole resistance, although further studies are needed to confirm its role.

Discussion and Conclusion

This study provides the first fully resolved genome of *M. clavatus*. The chromosomal organization is similar to *M. capitatus*. Phylogenetic analysis suggests the presence of a previously unrecognized lineage (clade D). The findings confirm the pathogen intrinsic echinocandin resistance due to mutations in the 1,3-beta-glucan synthase complex, while fluconazole resistance may be linked to modifications in the sterol 14-alpha demethylase enzyme. Further research is required to explore the functional impact of these mutations to develop new avenues for effective diagnostic and therapeutic strategies.

181 - In vitro evaluation of novel antifungal agents against clinical Candida spp. isolates

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In vitro evaluation of novel antifungal agents against clinical *Candida* spp. isolates

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1. Introduction. Fungal infections caused by *Candida* spp. are an increasing threat in hospital settings. The rapid growth of antifungal resistance correlates with a reduction in effective therapeutic options, highlighting the need for new promising alternatives. In this study, we compared the *in vitro* activities of three novel-generation molecules Rezafungin (RZF), Ibrexafungerp (IBX) and Fosmanogepix (FMGX) with those of Anidulafungin (ANID), Amphotericin B (AMB) and Fluconazole (FLU) against different *Candida* spp. isolates.

2. Materials and Methods. 72 clinical *Candida* spp. isolates were tested (13 *C. glabrata*, 12 *C. parapsilosis*, 4 *C. auris*, 20 *C. albicans*, 15 *C. tropicalis*, 8 *C. krusei*). MICs of antifungals were

determined by broth microdilution according to EUCAST guidelines. Time-kill kinetics of the new antifungals compared to AMB were assessed using concentrations at 1xMIC and 2×MIC.

3. Results. Four isolates of *C. glabrata*, six of *C. parapsilosis*, four of *C. tropicalis* were resistant to FLU and all *C. auris* isolates exhibited high values (>16 µg/ml). Resistance to ANID was observed in two isolates of *C. glabrata*, one of *C. albicans*, one of *C. tropicalis* while *C. auris* isolates exhibited MIC values ranging from 0.03 to 0.016 µg/ml. Six isolates of *C. krusei* were non-susceptible to AMB and three *C. auris* isolates demonstrated an MIC > 4 µg/ml for AMB. FMGX MICs ranged from 0.001 to >128 µg /ml. MIC_{50} showed a rank order of susceptibility with *C. parapsilosis* (0.004 µg/ml) ≈ *C. auris* (0.004 µg/ml) ≈ *C. albicans* (0.004 µg/ml) > *C. tropicalis* (0.008 µg/ml) > *C. glabrata* (0.06 µg/ml) > *C. krusei* (>128 µg/ml). IBX MICs ranged from 0.016 to 4 µg/ml. MIC_{50} showed a rank order of susceptibility with *C. albicans* (0.06 µg/ml) > *C. glabrata* (0.25 µg/ml) ≈ *C. parapsilosis* (0.25 µg/ml) ≈ *C. auris* (0.25 µg/ml) ≈ *C. tropicalis* (0.25 µg/ml) > *C. krusei* (0.5 µg/ml). RZF MICs ranged from ≤ 0.002 to 1 µg/ml. MIC_{50} showed a rank order of susceptibility with *C. albicans* (≤0.002 µg/ml) ≈ *C. tropicalis* (≤ 0.002 µg/ml) > *C. auris* (0.008 µg/ml) > *C. glabrata* (0.016 µg/ml) ≈ *C. krusei* (0.016 µg/ml) > *C. parapsilosis* (1 µg/ml). The 24-hour time-kill assays showed that RZF and IBX exhibited a trend comparable to that of AMB for most of the tested strains, with a significant reduction in fungal growth observed at both 1×MIC and 2×MIC concentrations. FMGX demonstrated a concentration-dependent effect, with partial inhibition of fungal growth at 1×MIC and complete suppression at 2×MIC.

4. Discussion and Conclusions. The three next-generation antifungals demonstrated promising *in vitro* efficacy, even against species commonly difficult to treat. Further studies are needed to investigate their safety and efficacy *in vivo*.

185 - Concurrent Opportunistic Infections by Cryptosporidium spp. and Geotrichum capitatum in an Immunocompromised Patient with Acute Myeloid Leukaemia

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Concurrent Opportunistic Infections by *Cryptosporidium* spp. and *Geotrichum capitatum* in an Immunocompromised Patient with Acute Myeloid Leukaemia

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Introduction: Here we discuss the case of a 39-year-old woman with acute myeloid leukaemia (AML). The patient presented to the Emergency Department of the "Pugliese Ciaccio" Hospital in Catanzaro (Italy), two weeks after a dental procedure carried out in Albania. The patient was admitted with severe asthenia, intermittent fever, and gingival bleeding. During hospitalization, recurrent gingivorrhagia and syncopal episodes were observed. Blood tests revealed marked neutropenia, thrombocytopenia, and abnormal leukocyte count. Pathology evaluation of bone morrow confirmed the diagnosis of acute myeloid leukaemia and chemotherapy was initiated, thus leading to immunosuppression. Subsequent microbiological investigations identified *Geotrichum capitatum* in blood culture samples, an opportunistic fungal pathogen typically found in leukemic patients. Due to respiratory failure, the patient was transferred to the Intensive Care Unit (ICU) of "Mater Domini" University Hospital ("Renato Dulbecco" facility Catanzaro (Italy)). Materials and Methods: During the initial hospitalization, blood cultures were performed and tested positive for *Geotrichum capitatum*, identified by MALDI-TOF following Sabouraud agar culture. A chemical-physical stool examination revealed liquid, black stools with abundant mucus. A coproparasitological analysis was then performed, by immunochromatographic card and subsequently confirmed by microscopic observation following Ziehl-Neelsen staining. Results: The examination performed on the faecal sample by

immunochromatographic card revealed *Cryptosporidium* infection; this result was confirmed by microscopic examination. To date, the patient is hospitalized in ICU which antibiotic, antifungal and antiparasitic tailored therapy. Discussion and conclusions: Such coinfections identified can be a consequence of the patient's severe immunosuppressive condition. According to the literature, *Geotrichum capitatum* is present in the environment and might cause invasive fungal infections, which shows a mortality range of 57% to 90 % in patients with AML. In several cases among patients with AML, especially during period of profound neutropenia induced by chemotherapy may emerge such rare infection. *Cryptosporidium* spp. On the other hand, is an obligate intracellular protozoan parasite associated with infections of the intestinal epithelium. In these patients with compromised immune system, such as leukaemia patients *Geotrichum* and *Cryptosporidium* coinfection. represents a very common complication.

195 - Evaluation of the Potential Antifungal Activity of a Polyphenol-Containing Extract Derived from Olive Mill Wastewater.

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Evaluation of the Potential Antifungal Activity of a Polyphenol-Containing Extract Derived from Olive Mill Wastewater.

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Introduction: Olive oil production generates large amounts of a by-product known as olive mill wastewater (OMWW). The uncontrolled disposal of OMWW poses serious environmental concerns. The aim of this study was to evaluate the antifungal activity of a dry extract obtained from OMWW of Cerasuola olives (Partinico, Palermo), rich in polyphenolic compounds, against *Candida albicans*. Specifically, the study focused on assessing the ability of the extract components to inhibit germ tube formation, a process closely associated with the pathogenicity of *C. albicans*. This activity has already been reported for hydroxytyrosol (HT).

Materials and Methods: The extract was subjected to biochemical characterization by High-Performance Liquid Chromatography (HPLC) to identify and quantify the major phenolic compounds. Hydroxytyrosol was found to be the most abundant compound in the extract. At lower concentrations, p-coumaric acid, cinnamic acid, caffeic acid, quercetin, and mandelic acid were also present. For antifungal testing, *C. albicans* cultures were grown on Sabouraud Dextrose Agar and incubated at 37 °C for at least 24 hours. The germ tube inhibition assay was performed by incubating 100 µL of a 4 McFarland *C. albicans* suspension in normal saline with 900 µL of fetal bovine serum (FBS) containing the extract at a concentration of 10 mg/mL. A control sample without the extract was also included. Samples were incubated at 37 °C for 24 hours and then microscopically examined to evaluate germ tube and blastospore formation.

The Minimum Inhibitory Concentration (MIC) was determined using a 96-well microplate, with concentrations ranging from 25 mg/mL to 3.15 mg/mL.

Results: Microscopic observation after only 2 hours of incubation revealed approximately 90% inhibition of germ tube formation in the samples treated with the extract compared to the control. A significant decrease in turbidity was observed only in the well with the highest concentration of the molecule.

Discussion and Conclusions: The results indicate that the polyphenol-rich extract possesses germ tube-inhibiting activity. This effect may be exploited to reduce the adhesive capacity of *C. albicans*, which is strongly associated with germ tube formation.

201 - The role of the S862C amino acid substitution in CpMrr1 in the development of azole resistance in Candida parapsilosis strains isolated from a clinical outbreak.

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The role of the S862C amino acid substitution in CpMrr1 in the development of azole resistance in *Candida parapsilosis* strains isolated from a clinical outbreak.

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Background. *Candida parapsilosis* is an emerging opportunistic pathogen with increasing rates of fluconazole- and voriconazole-resistance [1]. The most prevalent azole-resistance mechanisms are associated with the presence of amino acid substitutions such as the Y132F and G458S in the drug target lanosterol-14α-demethylase codified by the *CpERG11* gene, followed by mutations in the *CpMDR1* and *CpMRR1* and genes, encoding for efflux-pump and related transcription factor [2]. Recently, in an outbreak at Azienda Ospedaliero-Universitaria Pisana a new aminoacidic substitution S862C (C2585G) in the *CpMRR1* gene was found to be present only in azole-resistant strains. The contribution of this mutation was investigated in this study.

Methods. Antimicrobial resistance in *C. parapsilosis* clinical isolates (n=16) was tested with broth microdilution methods and E-test strip. Whole Genome and Sanger Sequencing analyses were used for the detection Single Nucleotide Polimorphisms (SNPs). Sanger sequencing was applied to investigate the presence of both mutations in adjunctive clinical strains retrieved in different time periods during the clinical outbreak from 2021 to 2024. A CRISPR-Cas9 based genome editing strategy was used to induce the C2585G substitution in the *CpMRR1* gene of *C. parapsilosis* susceptible isolates to investigate its role in the development of azole-resistance [3].

Results. Fluconazole- and voriconazole-resistance was found in 13 out of 16 clinical isolates along with the presence of the C2585G nucleotide substitution in the *MRR1* gene in all resistant isolates, but not in the susceptible ones. Adjunctive 57 resistant clinical strains were evaluated at Sanger sequencing and revealing the presence of the S862C and the Y132F substitutions in 52/57 (91.3%) and 55/57 (96.5%), respectively. Such mutation was later induced in the *C. parapsilosis* reference strain ATCC 22019 (Figure1) and in 2 susceptible clinical strains. Both heterozygous and homozygous mutants resulted to be fluconazole-resistant, with some clones presenting also intermediate susceptibility or resistance to voriconazole, and all showing an increase in the levels of RNA transcripts compared to parental strains (Table 1). At growth curves analyses heterozygous clones presented an almost overlapping growth rate compared to parental strains while homozygous clones revealed a slowed growth rate (Figure2).

Conclusion. To the best of our knowledge, this is the first study to report the effect on azole-resistance of a novel C2585G nucleotide substitution in the *CpMRR1* gene found in clinical isolates recovered during an outbreak of azole-resistant *C. parapsilosis* in a health-care setting.

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242 - Nuclear transport inhibitors as antileishmanial drug candidates: phylogenetic analysis, structural modelling, and in vitro antiparasitic assays

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Nuclear transport inhibitors as antileishmanial drug candidates: phylogenetic analysis, structural modelling, and in vitro antiparasitic assays

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Introduction

Leishmaniasis is a vector-borne, neglected tropical disease that affects millions of people worldwide. The severe toxicity of existing therapies, coupled with the increasing emergence of drug resistance, underscores the urgent need for new, safer treatment strategies. Nuclear-cytoplasmic transport inhibitors (NCTIs) represent a novel class of compounds that target cellular pathways not affected by current antileishmanial drugs. This study investigated their potential as anti-*Leishmania* agents by integrating phylogenetic analysis, structural modelling, and in vitro antiparasitic assays.

Materials and Methods

Orthologues of well-characterized mammalian nuclear transport proteins, including importin (IMP)alpha, IMPbeta1, and CRM1, were analyzed across *homo sapiens* and *Leishmania* species using Clustal Omega to construct phylogenetic trees and identity matrices. Structural similarity between parasitic and human protein homologues was assessed using Swiss-PdbViewer. The activity of selected NCTIs against *Leishmania infantum* and *L. donovani* was evaluated using the AlamarBlue assay for extracellular promastigotes and by microscopy examination of infected macrophages for intracellular amastigotes. Cytotoxicity was assessed in a human macrophage cell line, to calcolate the selectivity index (SI).

Results

Sequence analysis revealed high conservation among Leishmania nuclear transport proteins (92.2–96.7% identity) but significant divergence from their human counterparts (29.1–38.6%). Structural modelling, however, suggested substantial three-dimensional similarity between parasite and human proteins, raising the possibility that NCTIs could effectively target *Leishmania*. Among seventeen NCTIs tested *in vitro*, the IMPbeta1 inhibitor importazole (SI = 24) and the CRM1 inhibitor piperlongumine (SI = 5) selectively inhibited the growth of *L. infantum* and *L. donovani* promastigotes. Both compounds also displayed strong activity against intracellular amastigotes, without cytotoxicity to host cells.

Discussion and Conclusions

Although *Leishmania* nuclear transport proteins are highly divergent in sequence from human homologues, their structural conservation supports the feasibility of targeting them with NCTIs. Importazole and piperlongumine emerged as potent and selective inhibitors of *Leishmania* growth, highlighting NCTIs as promising candidates for novel antileishmanial drug development.

262 - Comparing methods for the diagnosis of Aspergillus spp infections: the experience of the Azienda Ospedaliera Universitaria Policlinico di Palermo.

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Comparing methods for the diagnosis of Aspergillus spp infections: the experience of the Azienda Ospedaliera Universitaria Policlinico di Palermo.

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Introduction:
Invasive aspergillosis (IA) is a serious, potentially life-threatening fungal infection that predominantly affects immunocompromised individuals. Early and accurate diagnosis of AI remains a clinical challenge. Galactomannan detection and molecular detection of Aspergillus spp. on bronchoalveolar lavage (BAL) allow rapid and sensitive diagnosis of infection, facilitating timely and targeted therapeutic interventions. The aim of our study was to evaluate the performance of rapid tests (antigenic test, PCR) in comparison to traditional culture examination in the diagnosis of AI infection.

Materials and Methods:
BAL samples from 20 patients with pneumonia refractory to antibiotic therapy were assayed, 5 of whom were admitted to the Pneumology Department and 10 to the Intensive Care Unit of the A.O.U.P. P. Giaccone of Palermo. The BAL samples were subjected to culture investigation, to the search for galactomannan antigen by Chemiluminescence method (Ag Virclia Alifax) and to nucleic acid research by Real-Time PCR (AB Analitica).

Results:
Of the 20 patients with suspected Aspergillus spp pulmonary infection, four tested positive in

both antigenic and molecular tests, four in the antigenic test only and two in the molecular test only. The culture test in only four patients detected growth of Candida spp, in the remaining patients there was no growth. Specifically, two of these were positive in both the antigenic and molecular tests, one in the molecular test alone, and one was negative in both tests.

Conclusions:
To date, rapid tests are a valuable aid for the rapid diagnosis of Aspergillus spp. infections, especially in high-risk patients. Our data, although preliminary, suggest greater accuracy in the diagnosis of suspected AI when using both tests. In conclusion, the sensitivities of the diagnostic tests evaluated when interpreted individually were often low but increased significantly when the diagnostic tests were combined.

03 3. Resistenza ai nuovi farmaci e implicazioni terapeutiche

17 - A simplified, fast and reliable checkerboard assay for antimicrobial combination studies

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A simplified, fast and reliable checkerboard assay for antimicrobial combination studies

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Introduction. The rise of multidrug-resistant pathogens has intensified the need for effective strategies to optimize existing antimicrobial agents, renewing interest in combination therapies to explore potential interactions. The checkerboard assay remains a gold standard for assessing antimicrobial interactions, yet traditional protocols are time-consuming, labor-intensive, and require large amounts of reagents and materials due to individual dispensing serial dilutions for each drug. To overcome these limitations, we developed a simplified method that improves operational efficiency while preserving methodological reliability. **Materials and methods.** The assay is performed in a 96-well microplate using three predefined concentrations of two antimicrobial agents. Drug dilutions are prepared in cation-adjusted Mueller-Hinton broth and dispensed with a multichannel pipette. After inoculation with a standardized bacterial suspension, plates are incubated at 35±2°C for 18±2h. Bacterial growth is measured by optical density at 600 nm using a microplate reader. Each plate includes 77 drug combinations, which are analysed for growth inhibition and interpreted using Loewe additivity and Bliss independence models. The protocol adheres to CLSI and EUCAST guidelines, with minor technical optimizations. **Results.** Compared to standard checkerboard protocols, the simplified method reduced preparation time and material consumption by over 50%. The protocol allowed the evaluation of 77 drug-drug combinations per plate, significantly minimizing reagent consumption and manual errors while improving workflow efficiency. The approach enabled consistent determination of both MIC values and interaction profiles. Testing of various antimicrobial combinations demonstrated reliable detection of synergistic, additive, antagonistic, and indifferent interactions, in line with standard criteria. **Discussion and conclusions.** The simplified checkerboard assay offers a reliable and cost-effective alternative to conventional methods for evaluating antimicrobial combinations. By significantly reducing preparation time, reagent use, and pipetting steps, the method enhances the

practicality of drug interaction testing, even in non-specialized or resource-limited laboratories. Its design enables rapid screening of 77 drug combinations per plate, including those involving non-antibiotic or repurposed compounds, facilitating the identification of synergistic, additive, antagonistic, and indifferent interactions. This approach supports both basic and translational research, bridging the gap between standardized microbiology protocols and high-throughput screening needs, and contributes to the development of novel strategies against multidrug-resistant pathogens.

19 - New life for old drugs: hydroxyl-benzoxaborole derivatives resensitise MRSA to oxacillin

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New life for old drugs: hydroxyl-benzoxaborole derivatives resensitise MRSA to oxacillin

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Introduction. Antimicrobial resistance is a global health crisis, driven by the rapid spread of multidrug-resistant pathogens. The new frontier of antimicrobial therapy aims to revert resistance by combining effective drugs to restore the activity of old or inactive molecules. Antimicrobial adjuvants in combination with available antibiotics are turning out the most promising approach. The study evaluated the adjuvant potentialities of hydroxyl-benzoxaborole derivatives, alone and in combination with oxacillin, against MDR methicillin-resistant *Staphylococcus aureus* (MRSA), also assessing their anti-biofilm activity. **Materials and methods.** Antimicrobial activity was tested by *in vitro* susceptibility test against MRSA ATCC43300. Synergy with oxacillin was assessed using checkerboard microdilution assays, interpreted by Loewe additivity (FICI) and Bliss independence (ΔE). Biofilm was qualitatively screened on Congo Red Agar and quantify by crystal violet staining in microplate assays. For inhibition tests, selected compounds (MIC ≤ 64 µg/mL) were tested at 1/8 to 4×MIC. Molecular docking of compound A was performed on the BlaR1 sensor domain using AutoDock4.0. **Results.** *In vitro* susceptibility assays revealed direct antimicrobial activity for a subset of derivatives, with compound A showing the lowest MIC value (16 µg/mL), followed by B,K, and L (64 µg/mL). Others showed no inhibition up to 128 µg/mL. Neverthless, all twelve compounds exhibited synergism when combined with oxacillin (FICI≤0.5). Notably, 11 out of 12 combinations restored oxacillin efficacy in MRSA ATCC43300 below the clinical resistance breakpoint (≤2 µg/mL). Selected compounds also inhibited biofilm formation, in some cases achieving complete (100%) suppression, supporting their role as adjuvants against biofilm-linked resistance. *In silico* analysis of compound A showed hydrogen bonds to BlaR1 sensor domain (Ser389, Thr527, Thr529), with a theoretical binding energy of -6.85 kcal/mol and

predicted *Ki* of 9.48 μM, suggesting potential interference with blaZ and/or mecA pathways. **Discussion/conclusions.** Hydroxyl-benzoxaborole derivatives emerged as promising multifaceted agents against MRSA. Beyond direct antimicrobial activity, all compounds synergised with oxacillin, drastically reducing its MIC and restoring susceptibility in a resistant strain. Their anti-biofilm effects enhance their potential in chronic infections. *In silico* data suggest a possible interference with early resistance signaling by targeting the BlaR1 sensor domain. These results position boronic derivatives as promising next-generation antimicrobial adjuvants in the fight against MRSA, especially relevant since structurally related boron-based drugs (e.g.,tavaborole, crisaborole, vaborbactam) are already FDA-approved.

20 - Probiotic Alkalihalobacillus clausii strains with broad-spectrum antimicrobial activities against Gram-positive bacteria: new frontiers in the era of antibiotic-resistance

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Probiotic *Alkalihalobacillus clausii* strains with broad-spectrum antimicrobial activities against Gram-positive bacteria: new frontiers in the era of antibiotic-resistance

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Introduction. Probiotic strains displaying natural antimicrobial activities are attracting increasing interest in clinical research, especially in the era of antibiotic-resistance. In this study, four strains of *Alkalihalobacillus clausii*, well-characterized for their probiotic properties, were investigated for their antimicrobial potential.
Materials and Methods. The antimicrobial activity of the four *A. clausii* strains was assessed by agar overlay assays against a plethora of Gram-positive, Gram-negative, and yeast reference strains and clinical isolates, both susceptible and resistant to antimicrobials. Culture supernatants of *A. clausii* strains were collected, fractionated, and concentrated, and the antimicrobial activity of each fraction was tested by broth microdilution. SDS-PAGEs were performed on each fraction to identify qualitative and quantitative differences in protein bands among the four strains. The isolation and identification of secreted molecules with antimicrobial activity are ongoing.
Results. *A. clausii* strains were active in solid media against all the tested Gram-positive bacteria, including *Actinomyces*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Listeria*, *Staphylococcus*, and *Streptococcus* spp. and regardless their resistance profiles. Interestingly, the four *A. clausii* strains exhibited different patterns of activity. No effects were observed against Gram-negatives and yeasts. SDS-PAGEs revealed no peculiar differences in terms of intensity/presence of protein bands among the four strains. The evaluation of antimicrobial activity of each fraction resulted in multiple molecules potentially involved in the overall activity and unidentified at present.
Discussion and Conclusions. The four *A. clausii* strains displayed broad antimicrobial activities against Gram-positive bacteria, including multidrug resistant microorganisms. Part of such activity may be explained by previous works focusing on a bacteriocin, but, since detected in different fractions and widely active against Gram-positives, their antimicrobial activities appear to be multifactorial. These findings have potential implications in the final use of such probiotics. Besides the secretion of beneficial molecules, such as short-chain fatty acids, vitamins, and antioxidant enzymes, these strains displaying antimicrobial activity may also be

intended for co-adjuvating the treatment of gastrointestinal Gram-positive pathogens, paving the way for alternative strategies to manage infections determined by multidrug resistant bacteria.

21 - Cyclodextrin-siderophore based drug delivery: the troyan horse strategy vs Escherichia coli

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Cyclodextrin-siderophore based drug delivery: the troyan horse strategy vs *Escherichia coli*

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Introduction. Antimicrobial resistance (AMR) represents a global challenge for public health, so it is necessary to find alternative strategies to fight bacterial infections. One of the most promising target is exploiting bacterial iron acquisition systems through siderophore-based drug delivery to increase intracellular drug concentrations: the Trojan horse strategy.
Materials and Methods. The study explored the deferoxamine B (DFO) and the gallic acid (GA) conjugates with beta-cicloextrins (CDs) as doxycycline (DX) carriers against *Escherichia coli* ATCC 9637 and *Staphylococcus aureus* ATCC 29213. The systems were characterized by NMR Spectroscopy and ESI-HR Mass Spectrometry (ESI-HR-MS) and their antibacterial activity was evaluated through Minimum Inhibitory Concentration (MIC) assay according to the CLSI and EUCAST guidelines. Moreover, the viability was assessed in the planktonic phase using the sub-MIC concentrations to determine the viable CFU/mL.
Results. The NMR confirmed the identity of the products and the formation of highly stable complexes with iron (III) with the conjugates. The +ESI-HRMS spectrum of CD funtionalized with GA showed the peak of molecular ion $[L+H]^+$ at m/z 1286.3988 and the single-charged sodium adducts $[L+Na]^+$ at m/z 1308.3988; CD funtionalized with DFO showed the peak ion at 1677.7083 m/z and double-charged peaks at 839.3586, 850.3475 and 858.3386 m/z corresponding to the $[L+2H]^{2+}$, $[L+H+Na]^{2+}$ and $[L+H+K]^{2+}$ species respectively. The lowest MIC value (0.125 µg/ml) was observed against *S. aureus* ATCC 29213, with no difference among CD added with DX funtionalized with GA or DFO and DX. The CD DFO derivative, enhanced the DX efficacy by lowering its MIC against *E. coli* ATCC 9637, exhibiting a MIC that was 2-fold lower (0.5 µg/ml) compared to CD funtionalized with GA and DX (1.0 µg/ml). CD funtionalized with

DFO and added with 0.250 μg/ml of DX showed the same inhibitory effect as 0.5 μg/ml DX tested alone. No significant effect was observed for CD funtionalized with GA tested added with 0.500 μg/ml DX.
Discussion and Conclusions. The beta-cyclodextrin conjugates with siderophore systems such as DFO and GA were synthetized and chemically characterized; the CD derivatives form iron (III) complexes showed promising effects as carriers for DX in bacteria allowing an increased DX antibacterial activity. Further studies are required to explore the mechanism of action of the CDs functionalized to assess their potential as a Trojan stragety.

24 - The UMIC Bruker® in testing Gram-positives in vitro susceptibility to dalbavancin: preliminary data on Staphylococcus spp. and Enterococcus spp. clinical isolates

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The UMIC Bruker® in testing Gram-positives *in vitro* susceptibility to dalbavancin: preliminary data on *Staphylococcus* spp. and *Enterococcus* spp. clinical isolates

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Introduction

Dalbavancin is a long-lasting lipoglycopeptide, which was approved for Gram-positive acute bacterial skin and skin structure infections treatment. Although the same mechanism of action of vancomycin, dalbavancin documented higher potency and half-life extension to 14 days, striving for reduced hospitalization and central venous accesses durations. Literature data reported dalbavancin off-label usage against *S. aureus,* coagulase-negative staphylococci and *Enterococcus* spp. endocarditis, pneumonia and bacteremia. Unfortunately, EUCAST guidelines require specific dilution conditions, complicating the dalbavancin integration within automated system for antimicrobial susceptibility testing. Our study evaluated the commercial microdilution Dalbavancin UMIC Bruker® in testing Gram-positive *in vitro* susceptibility.

Materials and Methods

We enrolled 16 *Staphylococcus* spp. (14 *S. aureus*; 2 *S. haemolyticus*) and 12 *Enterococcus* spp. (7 *E. faecalis*, 3 *E. faecium*, 2 *E. gallinarum*) clinical isolates, which underwent the UMIC Bruker® to gather dalbavancin MIC values, which were categorized according to EUCAST latest guidelines. The protocol registered MIC ranges, MIC50, and MIC90 values. Additionally, the collected results were compared to the reference EUCAST broth microdilution method, aiming to obtain essential agreement (EA) and categorical agreement (CA) percentages.

Results

All the *Staphylococcus* spp. strains reported susceptibility MIC values, with a 0.031-0.25 mg/L MIC range, a MIC50 of 0.125 mg/L and a MIC90 of 0.25 mg/L. These results revealed a 100% value both for EA and CA. *Enterococcus* spp. strains revealed MIC ranges of 0.063-0.25 mg/L together with a MIC50 of 0.125 mg/L and a MIC90 of 0.25 mg/L. Although a 67% EA, CA was not calculated due to the EUCAST breakpoint absence. However, *E. faecium* and *E. faecalis* gathered MIC values were equal or under the released epidemiological cut-off (E-COFF).

Discussion and Conclusions

Dalbavancin recently integrated numerous therapeutical protocols in the case of Gram-positives severe infections. Although its precision and reliability, reference broth microdilution require complex procedures and preparation for a laboratory diagnostic routine. We proposed the commercial RUO Dalbavancin UMIC Bruker® as a potential adaptation to provide MIC values within a conventional susceptibility workflow. The comparison to the broth microdilution showed interesting data, encouraging the dalbavancin susceptibility testing methods integration within clinical microbiology settings. According to clinical approaches and recent literature, dalbavancin may be an elective option in minimizing invasive central devices duration, prolonged hospitalization, and Gram-positive infective complications.

27 - The 50S mutations in linezolid-resistant Staphylococcus epidermidis, a growing concern in recent years

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The 50S mutations in linezolid-resistant *Staphylococcus epidermidis,* a growing concern in recent years

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Introduction: Linezolid is an oxazolidinone approved for the treatment of Gram-positive infections. Despite its recent introduction, antimicrobial resistance to this compound is

increasing worldwide. Gram-positive bacteria like *Staphylococcus epidermidis* are generally considered contaminants, but in some cases, they present linezolid resistance (LRSE) and cause nosocomial infections. In this light, fragile patients with complex clinical presentations are at risk. This study collected LRSE from 2017 to 2025 and analyzed five LRSE genomes to characterize mutations in *cfr*, 23S, and 50S genes. Material and Methods: 32 LRSE were collected from blood or cerebrospinal fluid (CSF) samples. Among these, the genome of 5 LRSE (3 from blood and 2 from CSF) was extracted and sequenced on Miseq Illumina Platform (Illumina Inc., San Diego, CA, USA). Reads were checked and trimmed, the genome was *de novo* assembled, and the 16S was used to confirm the identification. A core genome Multi-Locus Sequence Typing (cgMLST) on 1947 genes and ribosomal MLST (rMLST) were performed. The presence of the *cfr* gene was investigated, and the 23S was screened for mutations: G2576T, C2190T, T2502A, C2532T, and G2603T. Similarly, genes *rplC, rplD,* and *rplV* encoding L3, L4, and L22 proteins, respectively, were studied to detect mutations in the 50S locus. The resistance genes and plasmids were annotated through AMRFinderPlus v.3.12.8 and PlasmidFinder v.2.1.6. Results: The Minimum Inhibitory Concentration (MIC) resulted >4 for blood samples 1 and 4, >256 for blood sample 5; >256 for CSF samples 2 and 3. No *cfr* gene was found in any isolates, as well as mutations G2576T, C2190T, T2502A, and C2532T. The mutation G2603T was present in samples 2, 3, and 4. No mutation was found in *rplV* (L22); on the contrary, T467C was found in *rplC* (L3) of samples 3, 4, and 5. Additionally, mutations A347G and A407T were found in *rplC* of sample 3. In the sequence of *rplD* (L4) of sample 2, no mutations were annotated, whereas T99A was detected in samples 2, 3, 4, and 5. Samples 2, 3, and 5 presented the same set of mutations except for G295C that was present only in sample 5. Regarding the MLST analysis, samples 2, 3, 4, and 5 resulted in Sequence Type (ST) 2, on the contrary sample 1 was classified as ST190. According to the rMLST: sample 1 (rST-135820); samples 2 and 3 (rST-77178-rST181323-rST181324); sample 4 (rST-264034); sample 5 (rST-198009). According to the cgMLST, a difference of 2 alleles was found between samples 2 and 3 from CSF. Discussion and Conclusions: The study of genome mutations leading to linezolid resistance helps in monitoring the bacterial adaptation and arranging a prompt antibiotic treatment intervention. Particularly, all LRSE presented mutations in 50S.

31 - Interferon-beta enhances neutrophil microbicidal activity against Streptococcus pneumoniae and Klebsiella pneumoniae infections.

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Interferon-beta enhances neutrophil microbicidal activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* infections.

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Introduction: Our earlier research showed that both Gram-positive and Gram-negative extracellular bacteria trigger strong interferon-beta (IFN-beta) responses by activating endosomal Toll-like receptors that detect bacterial nucleic acids. The function of this cytokine during bacterial infections is complex and context-dependent. In this study, we examine how both endogenously produced and exogenously administered IFN-β influence the progression of infections caused by *Streptococcus pneumoniae* and *Klebsiella pneumoniae*, which are among the most common causes of community- and hospital-acquired pneumonia, respectively, and represent global health emergencies due to antibiotic resistance.
Materials and Methods: We investigated the role of endogenous IFN-beta by comparing infection outcomes in IFN-beta-deficient mice and wild-type controls, using a well-established pneumonia model. Additionally, the effects of recombinant IFN-beta (rIFN-beta) treatment

were assessed both in vivo and in vitro. Cytokine responses, NETs (neutrophil extracellular traps) and elastase release were measured by ELISA.

Results: The absence of endogenous IFN-beta significantly reduced the host's ability to combat *S. pneumoniae* and *K. pneumoniae* infections, as demonstrated by increased bacterial counts, reduced neutrophil infiltration, and lower levels of pro-inflammatory cytokines in the lungs of IFN-beta KO infected mice. These defects were reversed to wild-type levels following local administration of rIFN-beta. Furthermore, rIFN-beta enhanced resistance to both infections even in wild-type animals. The addition of rIFN-beta to bone marrow-derived macrophage and neutrophil cultures increased in vitro killing of both bacterial species. The effects on neutrophils were associated with enhanced NET formation and degranulation.

Discussion and Conclusions: Our findings demonstrate the protective role of both endogenous and exogenous IFN-beta in *S. pneumoniae* and *K. pneumoniae* infections. Notably, neutrophils were identified as the main responders to the beneficial effects of this cytokine, which were linked to increased production of NETs and antimicrobial proteins. These findings may be useful for the development of alternative strategies for treating infections caused by antibiotic-resistant bacteria.

51 - In-depth genomic analysis of azithromycin-resistant Neisseria gonorrhoeae isolates from laboratory surveillance in Italy (2020–2023)

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In-depth genomic analysis of azithromycin-resistant *Neisseria gonorrhoeae* isolates from laboratory surveillance in Italy (2020–2023)

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INTRODUCTION. The emergence of azithromycin (AZM) resistance in *Neisseria gonorrhoeae* is a global public health concern threating effective gonorrhea treatment. AZM resistance in *N. gonorrhoeae* has traditionally been associated with mutations in the 23s rRNA and in the *mtrRCDE* efflux pump system. Phenotypic and genomic investigations are crucial to monitor resistance to the first-line antibiotics and to guide therapeutic strategies. This study aims to investigate the genomic characteristics of AZT resistant in *N. gonorrhoeae* isolates (MICs >1 µg/mL) collected from 2020 to 2023 within the framework of the Italian laboratory surveillance for antimicrobial resistance in *N. gonorrhoeae*. Specifically, it focuses on genetic determinants—such as 23S rRNA and *mtrR* mutation—as well as on the identification of the most prevalent *Neisseria gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR) Sequence Types. **MATERIALS AND METHODS.** AZT susceptibility was determined using the MIC Test Strip method and interpreted according to EUCAST breakpoints. Whole-genome sequencing (WGS) was performed on a subsample of AZM-resistant isolates using the Illumina MiSeq platform. WGS data were used for *in silico* detection of molecular AMR determinants, including 23S rRNA and *mtrR*, and NG-STAR ST assignment through the publicly available PubMLST database (https://pubmlst.org/neisseria). **RESULTS.** Over the study period, gonococci showed low to moderate levels of AZM resistance (MIC values between 1.5 and 6 µg/mL) with mutations in the *mtrR* promoter region (either the -35A deletion or the *N. meningitidis*-like promoter sequence) but did not have mutations in the 23S rRNA gene. Conversely, isolates with moderate AZM resistance levels (MIC values between 4 and 32 µg/mL)

harboured both the C2599T mutation in the 23S rRNA gene, known to confer low to moderate resistance to AZM, and alterations in the *mtrR* promoter. The single isolate displaying a high-level resistance to AZM (MIC ≥256 µg/mL) harboured the A2143G mutation in the 23S rRNA gene—associated with high-level resistance—and a -35A deletion in the *mtrR* promoter. Overall, 26 NG-STAR Sequence Types (STs) were assigned to 61 of the 80 sequenced isolates; ST193 (n=10) and ST4463 (n=10) were the most frequently identified, mostly among isolates with low level of resistance. **DISCUSSION AND CONCLUSIONS.** In this study the AZT resistance patterns due to a combination of alterations in the *mtr* promoter and a specific mutation in the 23S rRNA gene, which together, or individually, contribute to low or moderate resistance to AZT were described. Ongoing phenotypic and genomic surveillance is essential to better understand the emergence of gonococcal resistant strains and their molecular mechanisms, helping to guide effective gonorrhoea treatment.

60 - Mimosa tenuiflora extract as an alternative to topic antimicrobials in venous ulcers

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***Mimosa tenuiflora* extract as an alternative to topic antimicrobials in venous ulcers**

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INTRODUCTION

In the management of vascular ulcerative lesions, it is essential to identify and, where possible, correct both macro- and microcirculatory disorders through medical and/or surgical interventions. The management of these lesions is challenging and really complicated, indeed there is a high risk of chronicity and development of infections, in some cases, resistant to the well-known antibiotic treatment. For this reason, there is the need of finding some valid alternatives, for example natural products that can help tissue repair and the prevention of microbial infections.

Among available therapeutic options, a plant-derived product obtained from the bark of *Mimosa tenuiflora* (also known as *M. hostilis*) has demonstrated significant healing properties. Rich in trace elements, tannins, and flavonoids, this compound has shown antimicrobial activity in both animal and human studies, particularly in the treatment of burns and vascular ulcers.

MATERIALS AND METHODS

Based on this background, we evaluated the efficacy of this extract in a cohort of outpatients with lower limb vascular ulcers of various etiologies, recruited in Elbasan, Albania. The study included 30 patients (15 males, 15 females), aged between 41 and 84 years. Following debridement, each patient received topical treatment twice daily for 30 days with an ointment containing *M. tenuiflora* extract (MTE). The ulcer surface area was measured by tracing the lesion outlines onto a millimeter-graduated plastic sheet at baseline and at the end of treatment. This method enabled the objective assessment of lesion evolution. Patients were evaluated twice weekly, throughout the treatment period, to monitor efficacy and identify any signs of intolerance or allergic reactions.

RESULTS

The ointment was well tolerated by all participants and exerted a favorable effect on wound healing, with clear improvement observed in 60% of cases and satisfactory outcomes in the

remaining 40%. Notably, no adjunctive antimicrobial therapy was required, due to the intrinsic antimicrobial activity of the extract. Healing was assessed by comparing lesion size before and after the treatment. From the first week, a significant reparative effect was noted, with progressive reduction in ulcer dimensions.

DISCUSSION AND CONCLUSIONS

MTE demonstrated therapeutic efficacy and excellent tolerability in all the treated patients, with no failure. Ulcer repair resulted from the synergistic action of flavonoids that stabilize cell membranes and inhibit pro-inflammatory mediators, and trace elements supporting enzymatic activity and cell adhesion. Antiseptic effects are due to tannins, demonstrating that MTE represents a valid alternative to the use of topical antibiotic in this condition.

61 - destructive interaction between electric fields and biofilm of multi-drug resistant Staphylococcus aureus.

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destructive interaction between electric fields and biofilm of multi-drug resistant *Staphylococcus aureus*.

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INTRODUCTION - Bacterial infections caused by multi-drug resistant bacteria, pose a serious threat to human health. These infections are often worsened by the formation of biofilm which interfere with the effectiveness of the immune response and antibiotic therapy. This study presents an experimental setup designed to investigate the effects of electric fields on mature biofilm of methicillin-resistant *Staphylococcus aureus* using Electrochemical Impedance Spectroscopy. The objectives were: *i)* to assess the impact of two different root mean square (RMS) electric field amplitudes (5 mV and 500 mV) over a frequency spectrum (10 Hz to 10 MHz), *ii*) to evaluate the effects of distinct exposure time, *iii*) to identify a narrower frequency range in which the electric field produces the most pronounced biofilm response.

MATERIALS AND METHODS – *S. aureus* ATCC 43300 biofilm was grown on PET slides in 1% glucose Brain-Heart infusion broth for 96 hours. Electrical stimulation included a Compensation Phase (CP) and a Test Phase (TP). Slides were divided into three groups based on three exposure time (2 min; 4 min; 6 min), with CP and TP cycles alternating to reduce system

drift and improve reproducibility. The biofilm response was evaluated through metabolic activity (XTT assay), biofilm biomass (crystal violet staining), viability (Colony Forming Units count), and biofilm structure (confocal microscopy) analysis.

RESULTS - A 500 mV electric field caused significant reductions in biomass, metabolic activity, and viable cell count, at all the exposure times. The 5 mV field reduced metabolic activity, biomass, and CFU counts in a time-dependent manner, with the most significant reductions occurring after the longest exposure time. Confocal microscopy observations confirmed the biofilm disruption. Additionally, three frequency subranges (SR) were tested (SR1=10 Hz to 1 kHz; SR2=1 kHz to 100 kHz; SR3=100 kHz to 10 MHz) with SR2 producing the highest reductions in bacterial biomass and CFUs.

DISCUSSION AND CONCLUSIONS - The study demonstrated that applying a low RMS voltage disrupts *S. aureus* biofilm, with the effect increasing both with exposure time and by raising the RMS voltage. Several hypothetical mechanisms for this biofilm reduction include increased membrane permeability, reduction of bacterial growth through oxygen generation, electrochemical production of oxidants, physical biofilm removal via electrolytic bubbles, and morphological changes to the biofilm. These findings highlight the potential of electric fields in biofilm disruption and will be a source of inspiration for further understanding of the mechanism of interaction between the electric field and biofilm, both in terms of treatment and diagnosis.

79 - Bacteriophage therapy in human infections: insights from a 5-year systematic review

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Bacteriophage therapy in human infections: insights from a 5-year systematic review

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INTRODUCTION:

Antibiotic resistance allows bacteria to survive inhibitory drug levels, posing a global threat. By 2050, over 39 million deaths could result from resistant infections. The rise of MDR and PDR bacteria demands urgent alternatives. Bacteriophages (phages), viruses targeting bacteria, offer a promising solution: they are species-specific, easy to isolate from the environment, and capable of reaching tissues often inaccessible to antibiotics, like the brain or bones. Interest in phage therapy is rising, especially for hard-to-treat infections.

MATERIALS AND METHODS:

A systematic review was conducted following PRISMA 2020 guidelines. PubMed and Scopus were searched with tailored queries to identify clinical studies on phage therapy (2020–2025). Only studies involving infected patients were included; animal studies, reviews, and non-English articles were excluded. The PubMed string used was: ("bacteriophage therapy"[Title/Abstract] OR "phage therapy"[Title/Abstract]) AND (clinical[Title/Abstract] OR "case report"[Title/Abstract] OR "case series"[Title/Abstract] OR "clinical trial"[Title/Abstract])NOTreview[Publication Type] AND ("2020/01/01"[Date-Publication]:"2025/12/31"[Date-Publication]).

For Scopus, the string used was: TITLE-ABS("bacteriophage therapy" OR "phage therapy") AND TITLE-ABS("clinical" OR "case report" OR "case series" OR "clinical trial") AND NOT DOCTYPE("re") AND PUBYEAR > 2020.

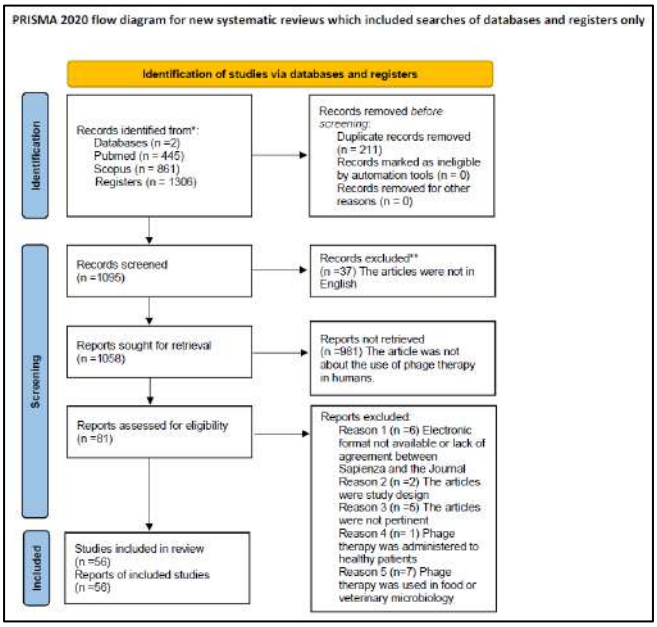

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Figure 1 PRISMA 2020 flowchart of the study selection process for the systematic review.

RESULTS:

Most studies involved respiratory infections. *P. aeruginosa* was the most common pathogen, followed by *K. pneumoniae*. Phage therapy often led to clinical improvement (85%) and microbiological eradication. In almost all cases, phages were combined with antibiotics, suggesting possible synergy, potentially lowering antibiotic MICs. Adverse effects were rare and mild (e.g., fever, elevated liver enzymes, anti-phage antibodies), without compromising efficacy. The impact on microbiota remains unclear, but phages' specificity likely minimizes disruption to normal flora.

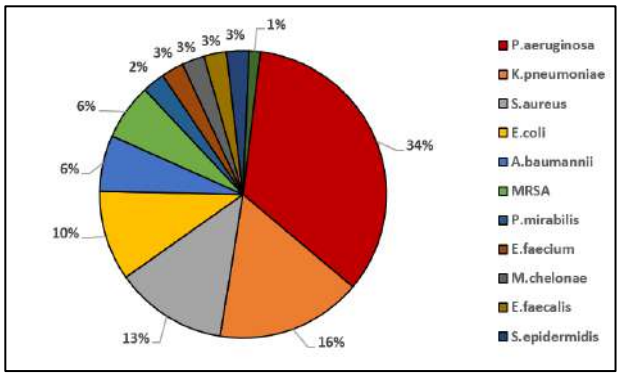

Figure 2 Percentage distribution of pathogens treated with phage therapy.

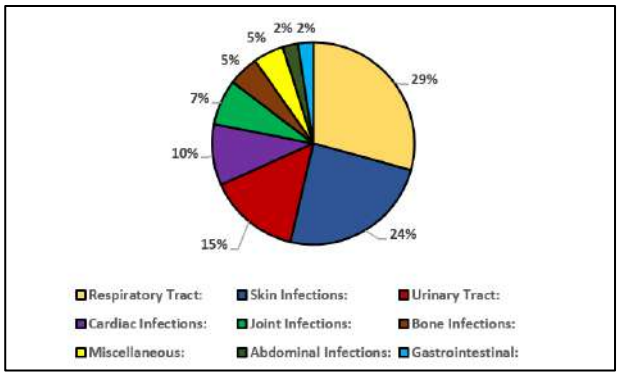

Figure 3 Distribution of infection sites in patients treated with phage therapy.

DISCUSSION AND CONCLUSION:

MDR and PDR infections require urgent alternatives to antibiotics. This review suggests phage therapy can improve outcomes and potentially enhance antibiotic efficacy. Despite encouraging results, phage therapy remains mainly used under compassionate use programs. Broader clinical trials are needed to validate its safety and efficacy.

89 - A workflow for environmental phage discovery and preliminary antibiotic synergy assessment targeting multidrug-resistant Acinetobacter baumannii

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A workflow for environmental phage discovery and preliminary antibiotic synergy assessment targeting multidrug-resistant *Acinetobacter baumannii*

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Introduction The threat of antibiotic-resistant bacteria calls for innovative therapeutic strategies like phage therapy via lytic phages. This study presents a proved "Phage Discovery" workflow addressing the current lack of standardized and scalable approach based on our experience with a multidrug-resistant (MDR) *Acinetobacter baumannii*. Our workflow included: phage isolation, characterization (comprising antibiotics synergy) and adaptation.
Material and Methods Clinically relevant bacterial strains were selected and used as hosts to isolate phages from wastewater. Following plaques formation, phages were visualized using transmission electron microscopy to assess morphological features and ensure clonal purity. Phage genome was sequenced using Oxford Nanopore Technologies, *de novo* assembled, annotated (*phold*), and evaluated for lifestyle prediction (*PhaTYP*). Matching prophage sequences were excluded by alignment with permissive host genome, previously sequenced using Pacific Biosciences. Lytic phage characterization was conducted via host range determination, functional assays, including thermal/pH stability, optimal multiplicity of

infection (MOI), and one-step growth curve. Moreover, phage-antibiotic synergy with cefotaxime, cefiderocol and meropenem was tested. Isolated phage was challenged to infect a previously characterized non permissive *A. baumannii* strain and adapted phage was re-characterized.

Results Among the phages isolated from wastewater using an *A. baumannii* host, one phage exhibiting *Siphoviridae*-like morphology (*Caudoviricetes*) and possessing a 79.9 Kbp genome was identified. *In silico* analysis predicted this phage to be lytic, with no detectable homology to the host genome. The phage exhibited high titer at 37°C and pH 7, with optimal MOI of 0.01. The lytic phage demonstrated a synergy with cefotaxime and cefiderocol**,** but not with meropenem. Adaptation to a non-permissive MDR *A. baumannii* strain was successful and stable over time, though no synergy with cefiderocol was tested as the strain was susceptible.

Discussion and conclusions Our workflow, combining long reads sequencing and prophage exclusion, successfully isolated and characterized a lytic phage against MDR *A. baumannii*. Preliminary assessment of synergistic interaction with cephalosporins supports the possible use of phages as adjuvant in antibiotic therapy. In conclusion, this scalable workflow may help mitigate common pitfalls, supports integrating phage therapy into antimicrobial strategies and building phage biobanks, offering new tools for the treatment of resistant bacterial infections.

Research supported by EU funding within the Next Generation EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, PE13 INF-ACT).

97 - The golden era: gold compounds for combating antibiotic-resistant staphylococcal infections

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The golden era: gold compounds for combating antibiotic-resistant staphylococcal infections

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1. Introduction: Today, infections caused by multidrug-resistant (MDR) staphylococci represent a critical public health challenge. Auranofin (AF), a gold-based drug approved for the treatment of rheumatoid arthritis, has been investigated for repurposing in the treatment of bacterial infections due to its promising antimicrobial activity. Herein, we investigated the anti-staphylococcal activity of AF and three new AF derivatives with the aim of discovering new warriors in the fight against antibiotic resistance. **2. Materials and Methods:** AF and three newly synthesized AF derivatives were used. Antimicrobial activity against reference and clinical strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*, characterized by different antibiotic resistance profiles, was determined by broth microdilution. Cytotoxicity on the A549 cell line was tested using the WST-1 colorimetric assay. The most promising compound, AF-Cl, was selected for further experiments. Killing kinetics were performed to assess the time course of bactericidal activity. The highest sub-inhibitory concentration of AF-Cl that failed to affect bacterial growth was used to test the ability of the compound to reduce *S. aureus* virulence by subjecting supernatants obtained from AF-Cl treated cells to hemolysis and agar diffusion assays. Further experiments investigating the anti-biofilm properties of AF-Cl are ongoing. **3. Results:** All compounds were active against staphylococci, with minimum inhibitory concentrations (MICs) ranging from 0.063 to 1 mg/L, and did not displayed cytotoxicity until 4-8 mg/L. Among the molecules tested, AF-Cl was identified as the most active, with MIC values ranging from 0.063 to 0.125 mg/L. This compound exhibited rapid bactericidal activity against clinical staphylococci, causing a 3-4 log reduction in the number of viable cells within 3-6 hours. When used at a sub-inhibitory concentration of 0.0156 mg/L, AF-Cl reduced the production of hemolysins, proteases, and phospholipases, which are key virulence factors in *S. aureus* infections. **4. Discussion and Conclusions:** Taken together, these results highlight the potential of AF-Cl as a promising candidate for the treatment of staphylococcal infections, including those caused by MDR strains. In addition, the compound showed anti-virulence properties, which may be beneficial in the treatment of toxin-mediated staphylococcal diseases.

Acknowledgments: *This work was Funded by the European Union - Next Generation EU within the National Recovery and Resilience Plan (PNRR) THE—Tuscany Health Ecosystem, Spoke 7—Innovating Translational Medicine—Sub-project 5—Innovative models for management of infections caused by antibiotic-resistant bacteria (Project code: ECS00000017; CUP I53C22000780001)*.

99 - Synergistic Combinations to enhance the activity of gold-compounds against Gram-negative bacteria

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Synergistic Combinations to enhance the activity of gold-compounds against Gram-negative bacteria

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1. Introduction: Multidrug-resistant Gram-negative (MDR-GNB) infections are a major threat to human health due to limited therapeutic options. Drug repurposing is an effective alternative strategy to address treatment challenges. In this context, recent studies have highlighted the antimicrobial potential of auranofin (AF), a gold-based drug approved for rheumatoid arthritis, particularly against Gram-positive bacteria. The aim of this study was to evaluate the antimicrobial activity of AF and its derivatives against Gram-negative bacteria and to explore potential synergistic combinations capable of enhancing their antimicrobial efficacy. **2. Materials and Methods:** AF and three AF derivatives (AF-Cl, AF-I, LMJA-01) were tested by broth microdilution against reference strains and clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Checkerboard assays were used to evaluate the potential synergy between the most promising derivative, AF-Cl, and polymyxin B (PMB), an antibiotic that alters outer membrane permeability. The interaction of AF-Cl with efflux pump inhibitors, such as carbonyl cyanide-m-chlorophenylhydrazone (CCCP) and berberine, was also evaluated to investigate the role of efflux pumps in resistance to AF-Cl. **3. Results:** AF and its derivatives were active against Gram-negative bacteria, with minimum inhibitory concentrations (MICs) ranging from 4 to over 64 mg/L. AF-Cl was the most active AF derivative

and strongly synergised with PMB against *P. aeruginosa*, with a fractional inhibitory concentration index (FICI) of 0.375. Combination with the efflux pump inhibitor CCCP also significantly increased the efficacy of AF-Cl against *P. aeruginosa*, whereas synergy with berberine was less pronounced. **4. Discussion and Conclusions:** The activity of AF-Cl against Gram-negative bacteria can be enhanced by combining the compound with PMB or efflux pump inhibitors, suggesting that both the barrier effect of the outer membrane and the activity of efflux pumps may account for the relatively low susceptibility of Gram-negative bacteria to the compound. The combination of an FDA-approved drug with the last-resort antibiotic PMB may offer perspectives for therapeutic interventions against MDR-GNB.

Acknowledgments: *This work was funded by the European Union-Next Generation EU within the National Recovery and Resilience Plan (PNRR) THE-Tuscany Health Ecosystem, Spoke 7-Innovating Translational Medicine-Sub-project-Innovative models for managing infections caused by antibiotic-resistant bacteria (Project code: ECS00000017; CUP I53C22000780001).*

108 - In vitro evaluation of the novel antibiotic zosurabalpin against carbapenem-resistant Acinetobacter baumannii clinical isolates

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***In vitro* evaluation of the novel antibiotic zosurabalpin against carbapenem-resistant *Acinetobacter baumannii* clinical isolates**

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Introduction

The increasing prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) represents a major global health challenge due to limited treatment options. Zosurabalpin (ZAB) is a novel tethered macrocyclic peptide antibiotic, developed by Roche (Switzerland). It targets the $LptB_2FGC$ complex, thereby blocking the transport of lipopolysaccharide (LPS) from the inner to the outer bacterial membrane. This study aimed to assess the *in vitro* activity of ZAB against CRAB isolates.

Materials and Methods

A total of 50 clinical CRAB isolates were collected from respiratory samples and blood cultures of hospitalized patients at University Hospital Policlinico Umberto I in Rome. These isolates were tested for their susceptibility to ZAB and cefiderocol (FDC). Identification of bacterial isolates was performed using MALDI-TOF MS (Bruker Daltonik, USA) and to determine whether the strains were CRAB, meropenem susceptibility testing was assessed using the MicroScan walkaway system (Beckman Coulter, USA). ZAB MICs were determined by broth microdilution according to CLSI guidelines, using homemade cation-adjusted Mueller-Hinton broth (CAMHB) supplemented with 20% heat-inactivated horse serum. Instead, antimicrobial susceptibility to FDC was tested using the microdilution method with homemade iron depleted (ID)-CAMHB. The concentrations of both antibiotics ranged from 32 mg/L to ≤ 0.015 mg/L. Clinical breakpoints for FDC and meropenem were defined according to the EUCAST 2025 guidelines.

Results

ZAB demonstrated potent *in vitro* activity against all clinical CRAB isolates, with an MIC_{90} value of 0.12 mg/L and MIC values ranging from ≤0.015 to 0.25 mg/L. Three of the 50 clinical isolates showed resistance to FDC, with MIC values of 4 mg/L, >32 mg/L, and >32 mg/L. Interestingly, these strains were susceptible to ZAB, with MIC values of 0.06 mg/L, 0.12 mg/L, and 0.06 mg/L, respectively.

Discussion and Conclusions

These preliminary findings indicate that ZAB exhibits strong *in vitro* activity against CRAB including strains resistant to last-line commercially available antibiotics such as FDC.

This research was supported by PAN_HUB T4-AN-07, Italian Ministry of Health, CUP B83C22004480001 to G. A.

111 - Comparison of cefiderocol susceptibility testing methods in multidrug-resistant Acinetobacter baumannii

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Comparison of cefiderocol susceptibility testing methods in multidrug-resistant *Acinetobacter baumannii*

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Introduction

Acinetobacter baumannii is a leading cause of severe hospital-acquired infections, especially in intensive care units (ICUs), and is often associated with multidrug resistance. In 2024, the WHO classified it as a pathogen with priority 1. Cefiderocol (FDC), a novel siderophore cephalosporin, represents a favourable therapeutic option. The appropriate susceptibility testing for FDC in gram-negative bacilli, particularly regarding its application to Acinetobacter spp., remains a matter of debate and generates considerable uncertainty. Specifically, its reference susceptibility testing method, broth microdilution (BMD) in iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB), is not feasible for routine use. This study aimed to evaluate alternative methods for FDC susceptibility testing in carbapenem-resistant *A. baumannii* (CRAB) clinical isolates.

Materials and Methods

Fifty CRAB isolates were isolated from respiratory samples of patients hospitalized in the ICU of 'Umberto I' academic Hospital in Rome. Microbial identification was performed using MALDI-TOF mass spectrometry (Bruker Daltonik, USA). FDC susceptibility was tested using homemade BMD in ID-CAMHB (reference method), commercial BMD-based systems (UMIC®

Bruker, USA and ComASP®, Liofilchem, Italy), gradient diffusion strips (MIC-test-strips®, Liofilchem, Italy) and disk diffusion (DD, Liofilchem, Italy). Results were analyzed in terms of category agreement (CA), essential agreement (EA), very major errors (VME), and major errors (ME) compared to BMD. The clinical breakpoints have been defined according to the EUCAST 2025 guidelines.

Results

Among the 50 isolates, BMD identified 47 susceptible strains. UMIC® and ComASP® detected 44 susceptible strains, while DD and MIC-test-strips® both identified 48. CA was highest for DD and MIC-test-strips® (98%), followed by UMIC® and ComASP® (90%). EA was moderate for UMIC® (76%) and ComASP® (72%), but low for MIC-test-strips® (34%). VME rates were 33% for both UMIC® and ComASP®, with ME rates of 8.5%.

Discussion and Conclusions

FDC represents a favourable therapeutic option against multidrug-resistant *A. baumannii* and accurate susceptibility testing is essential for its clinical efficacy. According to the EUCAST guidelines, BMD in ID-CAMHB, though complex, has to be considered the reference method. DD demonstrated excellent CA with BMD and is suitable for routine screening. UMIC® and ComASP® though providing MICs, showed high VME rates and, to our opinion, their use should be combined with DD for better reliability. MIC-test-strips® underestimated susceptibility in our experimental setting. This research was supported by PAN_HUB T4-AN-07, Italian Ministry of Health, CUP B83C22004480001 to G. A.

112 - THE GHOST ARMY IN HIV INFECTION

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THE GHOST ARMY IN HIV INFECTION

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Introduction: The Human Immunodeficiency Virus (HIV) infection is a significant global health issue, affecting millions worldwide. Combating its spread requires both behavioral and biomedical strategies. Although Highly Active AntiRetroviral Therapy (HAART) is crucial in managing HIV, it is not a cure since the infection can persist in a latent form within CD4+ T lymphocytes. Moreover, HIV invasion of the Central Nervous System (CNS) represents another major hurdle in disease management. To address these challenges, we propose an innovative approach for resistant HAART strains or HIV infection in the CNS. **Materials and methods:** Engineered vesicle (EVs) mimicking the membrane of human T CD4+ cells were produced using Microfluidic Technology (MF) with the NanoGenerator Flex M. The organic phase consisted in a lipid component containing soy L-α-phosphatidylcholine, cholesterol, and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl). The morphology of the EVs was analyzed using Field Emission-Scanning Electron Microscopy (FE-SEM) and Dynamic Light Scattering (DLS) with a ZetaSizer, measuring sizes, distribution and zeta-potential. Their potential toxicity was assessed with an MTT assay on Vero-76 and U937 cells. EVs ability to be internalized was determined by exposing cells to varying concentrations of EVs for different time points (10 minutes, 1 and 2 hours). The primary goal was the creation of EVs mimicking human T CD4+ surface; then, the incorporation of membrane receptors (CD4+, CCR5, CXCR4) into a synthetic bilayer is a key step. **Results:** The DLS analysis revealed a homogeneous population of EVs with an average diameter of about 495 nm and a Zeta potential of -39.5 mV. Functional assays on Vero-76 cells showed that the EVs didn't fuse with membranes at all tested concentrations and incubation periods. These findings suggest that the EVs are

biocompatible and suitable candidates for further applications. The successful incorporation and expression of receptors will be validated using Western Blot and ELISA. The ability of the modified EVs to traverse a synthetic Blood-Brain Barrier (BBB) model and reach the CNS will then be assessed. *In vitro* antiviral assays will evaluate the capacity of the receptor- EVs to bind and fuse with HIV in various cellular models. Finally, selected antiviral drugs will be encapsulated in the EVs lumen to enhance their activity via targeted delivery mechanisms. **Discussions and Conclusions:** This project proposes a promising strategy for combating HIV infection by developing an EV-based platform, having the potential to significantly address the limitations of conventional therapies and Next-Generation Strategies such as Kick and Kill, CRISPR-Cas9 editing, and CAR-T cell therapies.

117 - Antiviral activity of clays in epoxy composites: a new tool against healthcare associated infections

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Antiviral activity of clays in epoxy composites: a new tool against healthcare associated infections

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Introduction. Inanimate surfaces play a crucial role in the transmission of nosocomial pathogens with particular attention to High Touch Surfaces, (HTSs). To prevent the microbiological contamination of HTSs different technologies have been developed as alternatives to conventional cleaning. More recently, self-disinfecting surfaces with incorporated antimicrobial substances have been proposed. Epoxy resins have long been recognized as versatile materials with widespread applications due to their exceptional mechanical properties, ease of processing and tunable chemical properties that make them particularly suitable for preparing composites exhibiting improved characteristics, such as antimicrobial activity by integration with antimicrobial functionalities. Modified clays have been also considered.

Materials and methods. A commercial epoxy resin, MGS/RIMH235, which we had previously demonstrated having antiviral activity, was used to prepare nanocomposites by addition of different amounts of Cloisite 15A(C15A), an organo-modified clay with an ammonium salt bearing different aliphatic chains to promote compatibilization between the polymer and the clay. Preliminary results showed that composites based on this resin and different percentage (5%, 8% and 10%) of C15A demonstrated a significant antiviral activity using two experimental protocols: a) the virus suspensions were put in contact with the resins for different exposition times; b) they were exposed to buffer solutions in which the resins had been previously incubated for 24h. In both cases, at the end of the incubation time, the residual infectious titre was determined by end point titration. **Results.** Against Human Coronavirus OC-43, a virus infectivity reduction (>4 Log) was observed with composites containing 5% of C15A at 24h with both protocols; a modest antiviral effect (about 1 Log) was also observed for the nanocomposites with 1 and 3% of C15A. All the nanocomposites proved to be more active on HCoV-OC43 than the resin as it is. Under the same experimental conditions Herpes Simplex Virus-1 showed a lower sensitivity (approximately 2 Log reduction) and only for nanocomposite with 5% of C15A. It is to note that, with HSV-1, differently from what was observed with HCoV-OC43, the resin itself showed a more marked antiviral activity compared to the nanocomposite. Finally, no significant reduction of Adenovirus-5 infectivity was observed in any condition tested.
Discussion and conclusions. Epoxin resin based nanocomposites including clays may represent a useful tool in the fight against healthcare associated infections. These modified resins could be used to manufacture tools or surfaces with self-disinfecting properties in clinical settings.

119 - Towards Phage-Based Strategies for Pseudomonas aeruginosa Infections: Isolation and Functional Characterization from a Clinical Sample

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3. Resistenza ai nuovi farmaci e implicazioni terapeutiche

Towards Phage-Based Strategies for *Pseudomonas aeruginosa* Infections: Isolation and Functional Characterization from a Clinical Sample

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1. Introduction

Bacteriophages (phages) infect bacteria via lytic or lysogenic cycles. In the lytic cycle, phages replicate within the host, leading to bacterial lysis—a mechanism of increasing interest as an alternative to antibiotics against multidrug-resistant (MDR) pathogens. *Pseudomonas aeruginosa* is a clinically significant MDR bacterium, especially in cystic fibrosis (CF) patients. In this context, phages are gaining attention as precision tools for targeted antibacterial therapy, particularly in settings where antimicrobial resistance limits treatment options. This study aimed to characterise a phage from a *P. aeruginosa* isolated from a routine sputum sample processed at the Microbiology Laboratory of AOU Health and Science City of Turin.

2. Materials And Methods

A strain of *P. aeruginosa* isolated from sputum culture exhibited lysis-like plaques. The agar from the affected areas was excised, vortexed, centrifuged and the supernatant was filtered. The presence of phage was confirmed by double-layer agar (DLA) method. Following enrichment, the phage titer, thermal and pH stability, and morphology by Transmission Electron Microscopy (TEM) were evaluated. The optimal Multiplicity of Infection (MOI) was determined, and a one-step growth curve was performed to characterise the replication cycle. Host range was assessed on 30 clinical strains of *P. aeruginosa* isolated from different specimens and characterized for their resistance phenotypes.

3. Results

The phage formed clear plaques in DLA and reached a titer of 10^{14} Plaque Forming Units (PFU)/ml after enrichment. It remained stable (10^{14} PFU/ml) at 4°C, 20°C, and 37°C, while titers declined at -20°C (10^{7}), 50°C (10^{8}), and 70°C (10^{4}). At pH 7 and 10, stability was retained; pH 2, 3, and 12 led to inactivation, while pH 4 and 11 caused reduction (10^{9}). TEM revealed a *Myoviridae*-like morphology, typically associated with lytic activity. MOI 100 was optimal, yielding a titre of 10^{9} PFU/ml. The one-step growth curve showed a ~30-minute latent period, ~75-minute burst time, and burst size of ~13 PFU per infected cell. Host-range assays revealed susceptibility in 17 of 30 *P. aeruginosa* strains, including MDR isolates.

4. Discussion And Conclusion

The isolation of a phage from a clinical specimen represents a significant finding, suggesting *in vivo* presence and potential clinical relevance. It demonstrated thermal and pH stability, and a lytic morphology. Notably, the phage displayed optimal replication at a high MOI, which led to a rapid and synchronized infection cycle, as evidenced by its latency period and its burst time. Although the burst size was moderate, it may indicate a highly efficient replication in conditions of high bacterial density, as commonly found in chronic infections like those in CF lungs. Its ability to lyse most of tested clinical *P. aeruginosa* isolates, MDR strains included, underscores its therapeutic potential.

128 - Antibacterial properties of new green nanovesicles of benzalkonium chloride

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Antibacterial properties of new green nanovesicles of benzalkonium chloride.

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Introduction: The widespread use of benzalkonium chloride (BKC) in disinfectants and household products has raised concerns due to its associated drawbacks, including human toxicity and environmental contamination of water systems resulting from domestic, agricultural, industrial, and clinical discharge. Such contamination contributes to the growing issue of antimicrobial resistance. We propose co-encapsulating BKC and limonene (a natural essential oil with established antimicrobial activity) to address these challenges within nanovesicular systems. This strategy aims to reduce the required concentration of BKC while maintaining efficacy and aligning with the principles of green chemistry and sustainable development. **Materials and Methods:** We developed nanovesicles (NVs) loaded with benzalkonium chloride (BKC) at different concentrations (0.008% w/v, 0.08% w/v, and 2.4% w/v), either with or without a gaseous decafluoropentane (DFP) core. The formulations were characterized in terms of stability, encapsulation efficiency, release kinetics, and spray angle. Their antimicrobial activity was assessed by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *Staphylococcus aureus* and *Escherichia coli*, as well as through a quantitative suspension test following ISO EN 1040:2005 guidelines to evaluate the basic bactericidal activity of chemical disinfectants and antiseptics. Antibacterial efficacy was expressed as the $\log_{10}$ reduction factor (RF). **Results:** All formulations were able to encapsulate BKC with high efficiency (encapsulation efficiency ~94%). The size of the nanovesicles ranged from 120 to 500 nm, and all formulations remained stable at room temperature for at least two years. Compared to water, all NVs improved sprayability, with BKC-NVs at 0.08% w/v exhibiting the widest spray angle (66°), in contrast to water (60°) and free BKC solution (26°). The release kinetics of BKC from the NVs varied depending on its concentration; notably, BKC-NVs at 0.08% w/v ensured a rapid release, with 80% of BKC released within 2 hours. These NVs also showed significantly lower MIC and MBC values (0.19 µg/mL for *E. coli* and 0.023 µg/mL for *S. aureus*) compared to free BKC (6 µg/mL for *E. coli* and 1.5 µg/mL for *S. aureus*). In the suspension test, BKC-NVs at 0.08% w/v demonstrated the highest bactericidal activity, reducing colony-forming units (CFUs) by at least 5 $\log_{10}$ units for both bacterial strains, both in the presence and absence of organic contamination (BSA), at 15 minutes and 1 hour. **Discussion and Conclusion:** In conclusion, we developed a novel green formulation of BKC with disinfectant/antiseptic properties, exploiting materials of natural origin to reduce the concentration of possible environmental contaminants, and improving the antimicrobial activity on both Gram-positive and negative bacteria.

130 - Isolation and characterization of a novel Acinetobacter baumannii bacteriophage from sewage water

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Isolation and characterization of a novel *Acinetobacter baumannii* bacteriophage from sewage water

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Introduction. Antimicrobial resistance (AMR) is one of the major global health challenges of our time, with profound implications for human health. In 2019 alone, infections caused by AMR bacteria were responsible for 1.27 million deaths worldwide. Among the critical pathogens identified by the World Health Organization (WHO), *Acinetobacter baumannii* stands out as a significant threat. This opportunistic bacterium primarily affects patients in intensive care units (ICUs) and immunocompromised individuals. Its increasing resistance to carbapenems has further exacerbated the crisis. In response to these mounting challenges, phage therapy has gained attention as a novel and innovative complementary approach for addressing infections caused by multidrug-resistant bacteria.

Materials and Methods. This study utilized five diverse clinical isolates of *A. baumannii* to identify novel bacteriophages from sewage water collected in Leuven, Belgium, in January 2025. Standard protocols were followed for phage enrichment, purification, and production, including double-layer agar (DLA) assays and spot tests for titration. Host-phage dynamics were investigated using Clariostar assays. Genome sequencing was performed with Illumina, and structural characteristics of the phages were analyzed using transmission electron microscopy (TEM).

Results. The enrichment procedure resulted in the isolation of ten potential bacteriophages, which were subsequently characterized by DNA sequencing. Among these, four genomes exhibited identical lengths (32,309 bp) and a GC content of 38.85%. BLASTn analysis revealed 100% similarity among the four contigs, with complete alignment to *A. baumannii* genomes, hinting towards a temperate bacteriophage. This phage genome also showed limited sequence similarity to bacteriophages within the *Caudoviricetes* class (maximum query coverage of 36% and 85.62% identity). Further analyses using VipTree and VIRIDIC confirmed that the isolated

phage represents a novel genus and species, highlighting its genetic uniqueness. Phage.AI confirmed its lysogenic nature.

Discussion. Phage therapy is emerging as a promising solution to combat infections caused by antibiotic-resistant bacteria, particularly *A. baumannii*. The findings of this study demonstrate that the phage isolated is genetically distinct from other known bacteriophages and could offer potential for further optimization through genetic engineering. Such modifications, e.g. making it strictly lytic, could enhance its therapeutic properties, making it an effective tool for addressing antimicrobial resistance. Future studies will focus on exploring the therapeutic and enzymatic applications of this phage, paving the way for innovative strategies to manage resistant infections.

Table 1. Representative table of the five Acinetobacter baumannii strains examined in this study from the KU Leuven University database.

Species	host and plasmid designation	medium	optimum growth temperature	Remarks	Source	Date
Acinetobacter baumannii	RUH134	LB	37°C	reference strain of EU clone II	Military Hospital Nederoverheembeek, Dr. Jean-Paul Pirnay	21/03/2012
Acinetobacter baumannii	NCTC 13423	LB	37°C	'Iraqibacter'	Military Hospital Nederoverheembeek, Dr. Jean-Paul Pirnay	21/03/2012
Acinetobacter baumannii	RUH 875	LB	37°C	reference strain of EU clone I	Military Hospital Nederoverheembeek, Dr. Jean-Paul Pirnay	21/03/2012
Acinetobacter baumannii	LUH 5875	LB	37°C	reference strain of EU clone III	Military Hospital Nederoverheembeek, Dr. Jean-Paul Pirnay	21/03/2012
Acinetobacter baumannii	AcBa-P2-29	LB	37°C	HS patient isolate treated with phage	HS patient (patient2) treated with phage HS Prima	20/11/2024, BL

Figure 1. Growth curve of the RUH875 bacterium in the presence of the four putative isolated phages, obtained using the CLARIOstar assay.

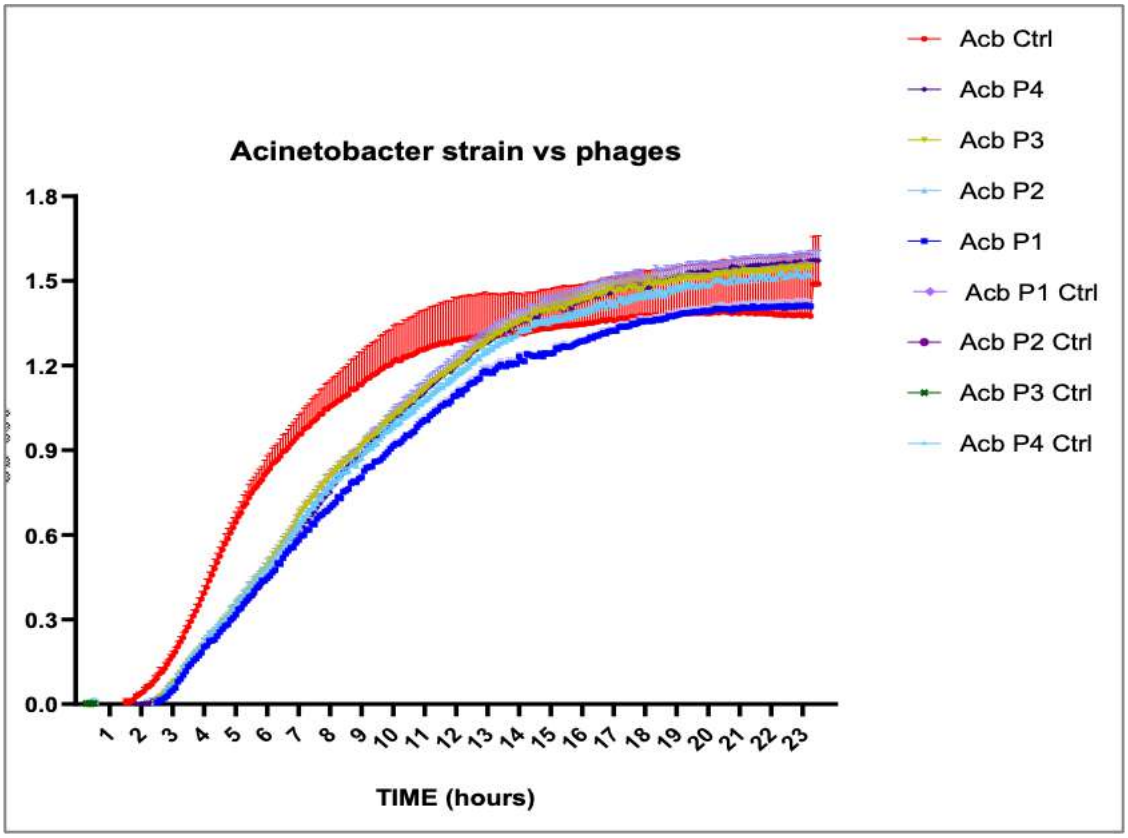


Figura 1. Growth curve of the RUH875 bacterium in the presence of a cocktail of phage, obtained using the CLARIOstar assay.

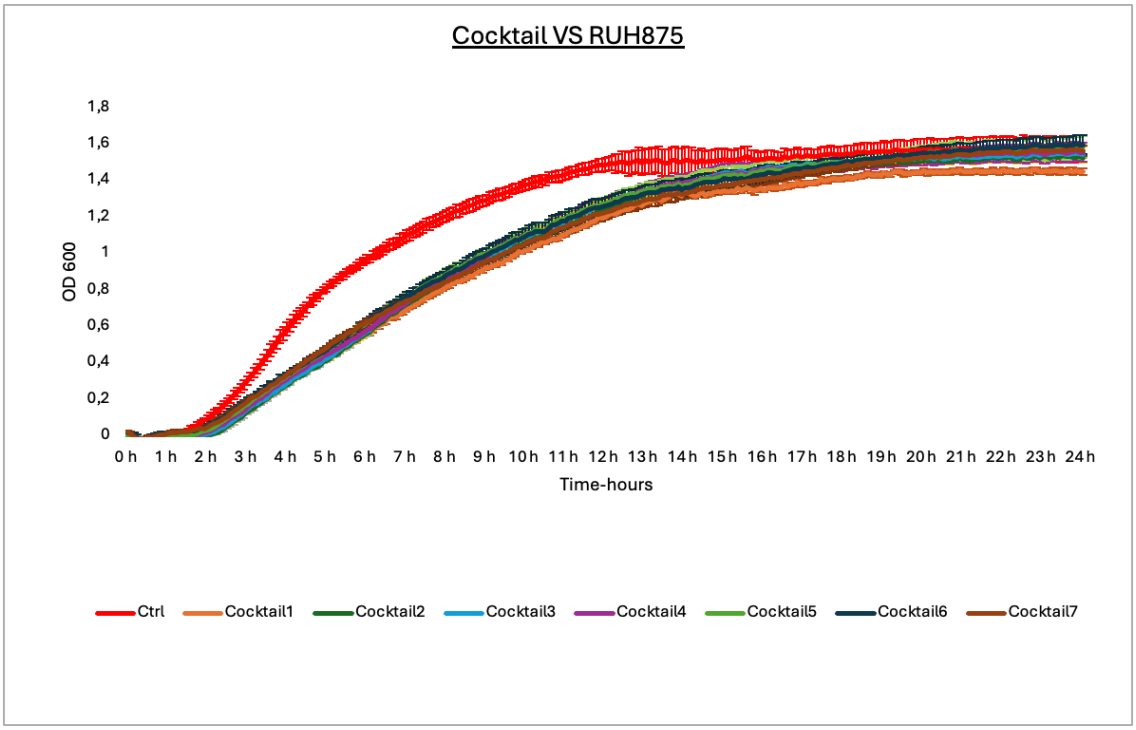


Table 2. Results of DNA quantification obtained using Qubit and Nanodrop.

Phages	Qubit (ng/ml)	Nanodrop (ng/ml)
Φ1	0.482	34.0
Φ2	/*	39.5
Φ3	0.260	32.1
Φ4	0.576	46.1

Figura 3. Phylogenetic tree of the novel phage obtained through sequencing and bioinformatics analysis.

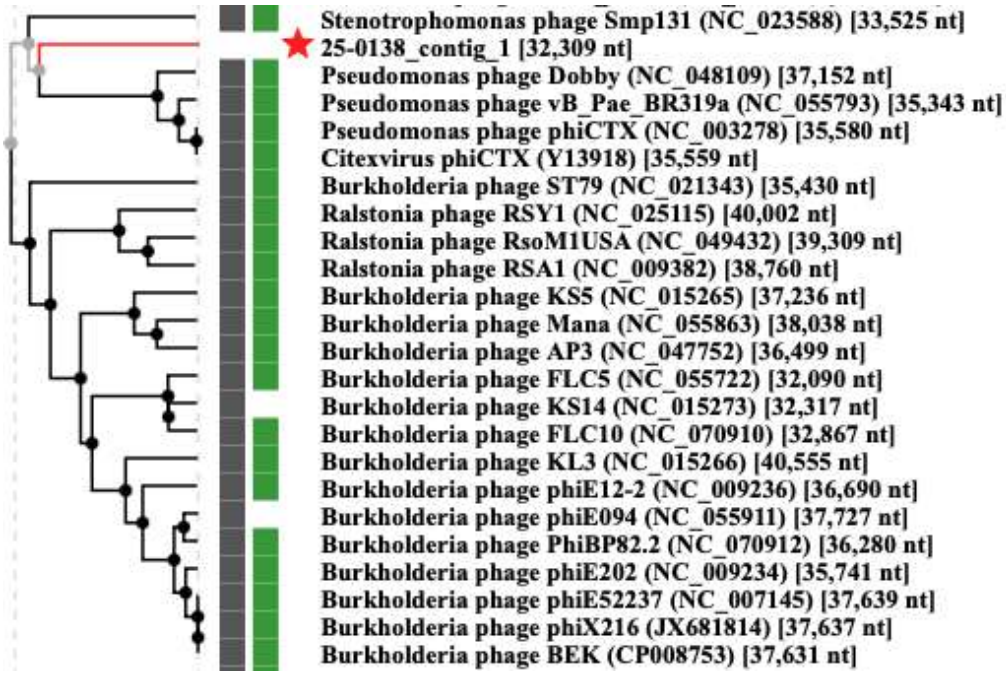
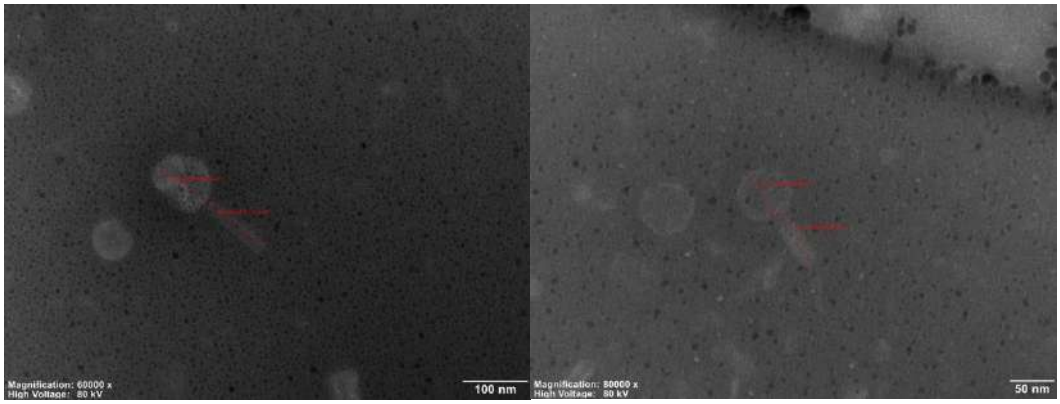


Figure 4. Two examples of images obtained using Transmission Electron Microscopy (TEM) of the novel bacteriophage, with the corresponding head and tail dimensions.



143 - Antibacterial and antibiofilm activity of a di-O-acylated deoxynojirimycin (DNJ) derivative against Staphylococcus aureus and Stenotrophomonas maltophilia.

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Antibacterial and antibiofilm activity of a di-O-acylated deoxynojirimycin (DNJ) derivative against *Staphylococcus aureus* and *Stenotrophomonas maltophilia*.

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Introduction: Iminosugars, such as deoxynojirimycin (DNJ), are sugar analogues well known for their glycosidase inhibitory properties and therapeutic potential in a wide range of diseases. These findings have inspired the search for novel derivatives, paving the way for this class of glycomimetics to emerge as promising therapeutic agents. In this study, a novel di-O-acylated DNJ derivative, 2,3-di-O-lauroyl-DNJ (compound **12**), was synthesized and biologically evaluated for its antibacterial and antibiofilm activity against gentamicin-resistant *Staphylococcus aureus* and amikacin-resistant *Stenotrophomonas maltophilia* strains.
Materials and Methods: The minimum inhibitory concentration (MIC) values of amikacin, gentamicin and compound **12** were measured by broth microdilution method, while the combination effects between compound **12** and antibiotics were performed by checkerboard assays. Biofilm inhibition and eradication of preformed biofilms were assessed by the crystal violet (CV) staining and the tetrazolium salt reduction (XTT) assays. Cytotoxicity was assessed using a red blood cells hemolysis assay.
Results: Although iminosugar **12** showed no antimicrobial activity against the strains tested (MIC > 2048 μg/mL), in the combination assay, the compound was able to enhance the antibacterial effects of gentamicin and amikacin against *S. aureus* and *S. maltophilia,* respectively. Gentamicin MICs were decreased up to 8-fold by combination with **12**. Similarly, the amikacin MICs were reduced 4-fold in the presence of the iminosugar **12**, from 32–128

μg/mL to 8–32 μg/mL. Compound **12** modestly inhibited biofilm formation, with the greatest effect against *S. aureus* strains with a 40% biomass reduction. However, the compound significantly reduced cell viability on preformed biofilms in a concentration-dependent manner. For *S. aureus* 00717, we observed a maximum reduction of 35%, in both biofilm biomass and metabolic activity. For *S. maltophilia* K279a, **12** at a concentration of 256 μg/mL significantly reduced biofilm viability by 55 %, although it had no significant effect on biofilm biomass. Finally, the hemolysis assay showed no cytotoxic effects on red blood cells.

Discussion and Conclusions: These results suggest that lipophilic modification of DNJ enhances its role as an antibiotic adjuvant by increasing bacterial susceptibility to aminoglycosides and targeting metabolically active cells within mature biofilm that are typically resistant to conventional antibiotics. The results open the way for structure-activity relationship (SAR) studies involving a variety of lipophilic iminosugars of the same class to optimize the observed antibacterial adjuvant effect.

151 - Bacteriophage Activity Against Biofilms of Ceftazidime/Avibactam-Resistant KPC-Producing Klebsiella pneumoniae

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Bacteriophage Activity Against Biofilms of Ceftazidime/Avibactam-Resistant KPC-Producing *Klebsiella pneumoniae*

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Introduction

Klebsiella pneumoniae producing KPC (Klebsiella pneumoniae carbapenemase) and resistant to ceftazidime/avibactam represents a major challenge for public health due to its ability to form biofilms, which enhance bacterial resistance to antibiotics and host immune responses. Bacteriophages, viruses that specifically target bacteria, have shown promise in disrupting biofilm structures, providing a potential therapeutic alternative. This study aimed to evaluate the anti-biofilm activity of bacteriophages isolated from hospital wastewater against clinical KPC-producing *K. pneumoniae* strains resistant to ceftazidime/avibactam.

Materials and Methods

Seven bacteriophages were isolated from hospital wastewater samples, each showing lytic activity against a specific clinical resistant strain. The biofilm-forming ability of *K. pneumoniae* strains (Kp8R, Kp10R, Kp26R, Kp27R, Kp28R, Kp5R, and Kp21R) was assessed in 96-well

microtiter plates. Strains were cultured in BHI (Brain Heart Infusion) broth at 37 °C until reaching an optical density (OD) at 600 nm (OD_{600}) of 1. Each well was inoculated with 180 µL of TSB (Tryptic Soy Broth) supplemented with 1% glucose and 20 µL of turbid cultures, and incubated for 24 hours. After washing with PBS (phosphate-buffered saline) to remove planktonic cells, bacteriophages were added. Biofilm biomass was quantified using 1% crystal violet staining, followed by washing, drying, and solubilization with 33% acetic acid. Absorbance was measured at OD600. The experiment was performed in triplicate, accompanied by three negative controls (lacking bacterial culture). The biofilm cut-off OD (ODc) was calculated as the mean OD of the negative controls minus the mean OD of the acetic acid wells. Strains were classified based on OD values as follows: nonadherent: OD < ODc, weakly adherent: ODc ≤ OD < 2xODc, moderately adherent: 2xODc ≤ OD < 4xODc, **strongly adherent:** OD > 4×ODc.

Results

Six of the seven strains demonstrated biofilm-forming ability. Phage treatment resulted in a significant reduction in biofilm biomass compared to untreated controls. One strain was classified as **nonadherent** and excluded from further analysis.

Discussion and Conclusions

Bacteriophages showed potential as alternative or adjunctive treatments against biofilm-associated infections caused by multidrug-resistant *K. pneumoniae*. Their high specificity and minimal impact on host microbiota support their clinical utility. Further molecular and clinical studies are necessary to assess their long-term efficacy, safety, and feasibility.

152 - Identification of the chemical composition of Origanum compactum essential oil and evaluation its antibacterial activity against aquaculture resistant pathogens

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Identification of the chemical composition of Origanum compactum essential oil and evaluation its antibacterial activity against aquaculture resistant pathogens

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Abstract

Over the past years, global production of fish from aquaculture has grown rapidly, contributing to significant quantities to the world's supply of fish for human consumption. Unfortunately, the sustainable expansion of this sector is currently hampered by a number of factors constraining fish production and development in terms of quantity and quality. Amongst those factors we find the intensification which leads to overcrowding and decrease water quality, facilitating the spread of pathogens and increasing disease outbreaks such as the emergence of microbial infectious diseases causing high mortalities within diverse fish species. To control the emergence of pathogens in aquaculture, antimicrobial agents are employed, but their overuse has lead to the increase of resistant bacteria that puts a huge pressure on aquatic

animal's health as well as consumers. Hence, in this work we aim to encapsulate the essential oil of *Origanum compactum*, then, identify its chemical composition using Gas chromatography coupled to mass spectrometry. Moreover, we aim to study its antibacterial activity against five fish pathogens in order to evaluate the capacity of encapsulated thyme essential oil to substitute antimicrobials for a further *in vivo* investigation in aquaculture as dietary supplements to increase fish immunity and growth performance.

Keywords: *Origanum compactum*, aquaculture, fish pathogens, antibiotic resistance, antibacterial activity.

159 - Synergistic interactions of levofloxacin with quercetin against Staphylococcus aureus and Pseudomonas aeruginosa biofilm

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Synergistic interactions of levofloxacin with quercetin against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm

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Introduction. The increased use of fluoroquinolones in ophthalmology has led to increasing resistance of *Staphylococcus aureus* and *Pseudomonas aeruginosa,* the most common pathogens associated with chronic keratitis linked to biofilm. An effective alternative therapeutic strategy can be the combinations of antibiotic and phytochemical having a synergistic activity and potential implication in delaying resistance. The aim of this study was to assay combinations of levofloxacin with quercetin against resistant biofilm forming strains. **Materials and Methods**. Levofloxacin (LVF) and quercetin (QUE) alone and in combinations

were tested against *S. aureus* and *P. aeruginosa* strains. Cytotoxic activity of QUE was evaluated by MTT method on human corneal epithelial (HCE) cells and by *Artemia salina* lethality bioassay. Antibacterial and antibiofilm activities were tested by microdilution techniques. The checkerboard assay was used to assess synergistic interactions between LVF and QUE both on planctonic growth and on biomass production of biofilm. **Results.** The MIC value of LVF was 0.122 µg/mL against *S. aureus* ATCC 6538 and *S. aureus* ATCC 43300 (MRSA) strains. The MIC values of antibiotic were 2 µg/mL and 250 µg/mL against *P. aeruginosa* ATCC 9027 and *P. aeruginosa* DSM 102273, respectively. QUE showed no activity against all strains at the maximum non-toxic concentration assayed. At ½ MIC LVF did not inhibit biofilm formation of all investigated strains, whereas QUE reduced biofilm biomass by 80% and 50% for *S. aureus* ATCC 6538 and MRSA, respectively. The combinations LVF with QUE showed synergistic interactions against planctonic cells of *S. aureus* ATCC 6538 with FIC index of 0.27 and a percentage of growth reduction of 63%, and of S. aureus ATCC 43300 with FIC index of 0.19 and growth reduction of 32%. The associations demonstrated additive interactions against *P. aeruginosa* ATCC 9027 and *P. aeruginosa* DSM 102273 with FIC index of 0.62 and 0.56, respectively. The combinations showed synergistic interactions against biofilm biomass of *S. aureus* strains with FIC index of 0.16, and of *P. aeruginosa* ATCC 9027 and *P. aeruginosa* DSM 102273 with FIC index of 0.37 and 0.07, respectively. **Discussion and Conclusions.** The synergistic results obtained demonstrated as the combinations of LVF with QUE improve the activity of LVF against the planctonic growth and biomass production of biofilm of investigated strains. Further studies are underway to evaluate if the obtained synergistic concentrations loaded in Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) can further enhance LVF antibiofilm activity.

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166 - Exploring the antimicrobial potential of WMRK-gH625: a new hybrid peptide against multi-drug resistant pathogens

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Exploring the antimicrobial potential of WMRK-gH625: a new hybrid peptide against multi-drug resistant pathogens

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Introduction: The emergence and rapid spread of Multi-Drug-Resistant (MDR) pathogens pose a significant threat to global public health, undermining the efficacy of conventional antibiotics. Antimicrobial peptides (AMPs) have gained considerable interest as alternative therapeutic agents due to their broad-spectrum antimicrobial activity, reduced risk of resistance development, and potential immunomodulatory effects. Among them, the WMR peptide (WMRp), a natural AMP derived from the marine hagfish, has demonstrated significant antimicrobial efficacy against Gram-positive and Gram-negative bacteria. Meanwhile, the cell-penetrating peptide gH625, originating from glycoprotein H of *herpes simplex virus type I*, is known for its ability to facilitate the intracellular delivery of various bioactive compounds, including macromolecules. This study aimed to evaluate the *in vitro* antimicrobial properties of a novel chimeric peptide WMRK-gH625 (WMRK-gHp), against both reference and clinical strains of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enterica*. **Materials and Methods:** The antimicrobial activity of WMRK-gHp was assessed through the broth microdilution assay to determine the Minimum Inhibitory Concentration (MIC). Time-Kill kinetic assays were employed to assess both bacteriostatic and bactericidal effects. To evaluate the safety profile, *in vitro* hemolysis assays were conducted on human erythrocytes, while cytotoxicity was examined in HeLa cells using MTT viability assays. Additionally, the impact of WMRK-gHp on oxidative stress was assessed by quantifying intracellular reactive oxygen species (ROS) production and finally the ability of peptides to induce membrane leakage was evaluated.
Results: WMRK-gHp exhibited a consistent bacteriostatic effect against all tested bacterial strains. Moreover, the peptide showed no cytotoxic effects in HeLa cells and no hemolytic activity in human erythrocytes. A significant reduction in oxidative stress was also observed following peptide exposure, indicating a potential *in vitro* protective effect. **Discussion and Conclusions:** The hybrid WMRK-gHp peptide demonstrated promising *in vitro* antimicrobial efficacy against key Gram-positive and Gram-negative MDR pathogens, along with a good safety profile in human cell lines. These findings support its potential as a lead compound for the development of novel antimicrobial agents targeting MDR infections.

177 - The Burden of Antimicrobial Resistance: In-vivo and Omic Fitness Costs in High-Risk MRSA Acquiring Reduced Susceptibility/Resistance to Glycopeptides and Daptomycin

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The Burden of Antimicrobial Resistance: In-vivo and Omic Fitness Costs in High-Risk MRSA Acquiring Reduced Susceptibility/Resistance to Glycopeptides and Daptomycin

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Introduction: The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to first-line glycopeptides (GLY) and last-resort antimicrobials such as daptomycin (DAP) poses significant challenges in clinical settings. Although antimicrobial resistance (AMR) allows the surviving under therapeutic pressure, it often comes with Fitness Costs (FCs) impacting growth, competitiveness, and virulence. Our goal was to investigate the in-vivo and omic FCs emerging with the stepwise acquisition of the DAP and GLY resistance (-R) and FCs in high-risk MRSA clones.

Materials and Methods: Three isogenic MRSA strain-pairs -ST5 N315 hospital-acquired-MRSA (HA-MRSA), ST398 livestock-acquired-MRSA (LA-MRSA), and ST1 MW2 community-associated-MRSA (CA-MRSA)- were investigated for FCs at the phenotypic level by independent growth, competitive mixed-growth, along with in-vitro and in-vivo virulence in *Galleria mellonella*, and at the omic level by a focus on essential gene (EGs) and virulence gene (VGs) genomics and transcriptomics by Illumina WGS, RNA-seq, and Bioinformatics analysis.

Results: ST5 N315 DAP-R HA-MRSA showed a very low in-vivo FC burden due to a low virulence-reduction and slow growth, as well as a very low competitiveness-burden. The ST398 DAP-R LA-hGISA showed a low in-vivo FC burden for a low virulence- and competitiveness-burden associated with a medium growth-one. The ST1 MW2 DAP-R CA-GISA showed a high in-vivo FC burden due to a huge virulence-reduction coupled with a high growth-retardation and competitiveness-reduction. At the omic level, comparative genomics evidenced moderate impact nsSNPs in EGs (such as *rpo*B, *rpo*C) as well as transcriptomics revealed differential expression and key dysregulations, mainly impacting the protein synthesis and cell-envelope EG-clusters, as well as the Virulome.

Discussion and Conclusions: In high-risk HA-, LA-, and CA-MRSA strains, the stepwise emergence of GLY-RS and DAP-R determines increasing FCs and key EG and VG "compensatory mutation and transcriptional-shift" adaptations.

198 - A Retrospective Study of Clinical Isolates: Antimicrobial Resistance in Enterobacteriaceae

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A Retrospective Study of Clinical Isolates: Antimicrobial Resistance in Enterobacteriaceae

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Introduction: Enterobacteriaceae are part of the human and animal microbiota and can become opportunistic pathogens. They are often involved in nosocomial infections such as bacteriemia, urinary infections and pneumonia. *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* are among the most clinically relevant species due to their ability to develop antibiotic resistance, often via the production of extended spectrum beta-lactamases and carbapenemases. Today, the increase of antimicrobial resistance is becoming a serious threat to public health. This retrospective study has analyzed the resistance profiles of

Enterobacteriaceae isolated from clinical samples collected from 2019 to 2023 at the *San Pio Hospital*, Vasto (Chieti), focusing on *E. coli, K. pneumoniae* and *P. mirabilis*.

Materials and Methods: Biological samples collected between 2019 and 2023 were analyzed at the Microbiology Laboratory of *San Pio Hospital*, following standardized procedures. Samples mainly included urine cultures (79.2%), blood cultures, swabs (vaginal, cutaneous, auricular, urethral), respiratory secretions, pus, biopsies and seminal fluid. After collection, the samples were transferred to selective agar media such as Columbia blood agar, MacConkey agar, Schaedler Vancomycin agar and Sven Gard agar, and incubated under aerobic or anaerobic conditions between 18 - 48 hours. Microorganism identification and antimicrobial sensitivity assessment were performed using the automated MicroScan WalkAway 40 Plus system, with PBC 32, NBC 81, NBC 82 and NBC 83 panels. Statistical analysis was carried out with software SPSS v20.0.

Results: This retrospective study analyzed 890 biological samples, positive for Enterobacteriaceae, collected between 2019 and 2023. The patients had a mean age of 73 years; 41.6% were hospitalized. The most common isolated species were *Escherichia coli* (63.17%), *Klebsiella pneumoniae* (21.02%), and *Proteus mirabilis* (8.15%). All strains exhibited resistance to at least one antibiotic, and over 50% were resistant to more than four antimicrobials. The highest resistance rates were observed for third-generation cephalosporins, particularly cefotaxime (15.96%), followed by ampicillin (15.57%) and ciprofloxacin (13.18%), highlighting the high prevalence of multidrug-resistant strains.

Discussion and Conclusions: The present study confirms a high rate of antimicrobial resistance among Enterobacteriaceae, especially in hospitals. These results highlight the need to strengthen infection control strategies and update therapeutic protocols, supporting clinicians in the empirical choice of antibiotics and preventing the spread of multidrug-resistant strains.

204 - Retrospective Study: Antimicrobial Resistance in Microorganisms Isolated from Patients with Bacteremia

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Retrospective Study: Antimicrobial Resistance in Microorganisms Isolated from Patients with Bacteremia

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INTRODUCTION: BloodStream Infections (BSIs) are caused by the presence of microorganisms in the bloodstream. BSIs are often associated with sepsis, a public health problem with a mortality rate between 12% and 34%. BSIs can occur in conjunction with localised infections, and their outcome is strongly influenced by AntiMicrobial Resistance (AMR), which is constantly growing worldwide. In this context, this retrospective study has analysed the main microbial species responsible for BSI and their antibiotic susceptibility profiles to contribute to the improvement of therapeutic strategies.
MATERIALS AND METHODS: In this retrospective study, 558 blood samples were collected between 2019 and 2023 at San Pio Hospital in Vasto (Chieti, Italy) from patients with suspected sepsis and were analysed. The samples, collected according to the protocol from peripheral venous accesses or central catheters, were inoculated into vials for the detection of aerobic and anaerobic bacteria, then incubated in the automated BacT/ALERT 3D system. Positive samples were cultured on selective media and analysed by the MicroScan WalkAway system

for microbial identification and antibiotic sensitivity. Statistical analysis was conducted using IBM® SPSS Statistics v 20.0 software.

RESULTS: Most infections (91.1%) were caused by a single microorganism, while the remaining 8.9% was related to multiple bacterial species. The most commonly identified bacterial genera were *Staphylococcus* spp. (47.7%), *Escherichia* spp. (15.4%), *Klebsiella* spp. (12.2%), and *Enterobacter* spp. (7.6%). Among these samples, 89.4% of the bacteria detected showed resistance to at least one antimicrobial agent, while 77.6% were resistant to multiple drugs. The bacteria identified, highlighted the highest resistance to β-lactams (43.1%), followed by aminoglycosides (14.1%), macrolides (8.9%), quinolones (8.3%), lincosamides (2.1%), and rifamycins (2%).

DISCUSSION AND CONCLUSION: This retrospective study evidenced an increase in antimicrobial resistance among bacterial species isolated from blood cultures of hospitalised patients, highlighting a growing challenge in the treatment of hospital-acquired infections. Most BSIs were associated with at least one microorganism resistant to a minimum of one antimicrobial agent. Most of the bacterial species identified showed multidrug resistance. The highest levels of bacterial resistance were found against beta-lactams and aminoglycosides. The data collected could help clinicians develop treatment guidelines, based on microbiological analysis, to choose an appropriate treatment to manage BSIs and prevent the progression towards sepsis.

206 - In Vitro Antimicrobial Activity of Selected Essential Oils Against MDR Clinical Pathogens

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***In Vitro* Antimicrobial Activity of Selected Essential Oils Against MDR Clinical Pathogens**

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Introduction

Essential oils (EOs) are natural products derived from aromatic plants, known for their antimicrobial properties. EOs are gaining attention as potential alternative therapeutic agents against infections mediated by multidrug-resistant organisms (MDROs) due to the limited availability of therapeutic options. This study aims to evaluate the *in vivo* antimicrobial activity of several EOs against a collection of MDRO.

Materials and Methods

The tested EOs were from *Eucalyptus globulus*, *Lavandula hybrida*, *Malaleuca alternifolia*, *Pelargonium graveolens* and *Cinnamomum zeylanicum* (Azienda Agricola Casalvento, Località Casalvento 35, Castellina in Chianti, Siena*)*. The *in vitro* antimicrobial activity was tested using broth microdilution (ISO 20776-1:2019) starting from an emulsion in cation-adjusted Mueller Hinton Broth supplemented with 10% of Tween 80 (Sigma-Aldrich). The collection included both ATCC reference strains (N=5) and clinical MDRO isolates from the collection of the University of Florence (N=11) belonging to the following species: *Staphylococcus aureus* (N=2), *Enterococcus faecium* (N=1), *Enterococcus faecalis* (N=1) *Klebsiella pneumoniae* (N=2), *Escherichia coli* (N=1), *Proteus mirabilis* (N=1), *Pseudomonas aeruginosa* (N=1), *Acinetobacter baumannii* (N=1) *Stenotrophomonas maltophilia* (N=3), *Serratia marcescens* (N=1),

Achromobacter xylosoxidans (N=1), *Burkholderia cepacia* (N=1). The minimum inhibitory concentration (MIC) data were reported as v/v percentage of EO able to inhibit pathogens' growth.

Results

The antimicrobial activity of EOs varied significantly depending on the MDRO species and plant species. In general, all tested EOs demonstrated antibacterial activity, with MIC below 8%. In particular, *Cinnamomum zeylanicum* EO showed the lowest MIC values, which ranged between 0.007 and 0.50 % v/v against all tested MDROs, including *K. pneumoniae* producing KPC and NDM carbapenemase, methicillin-resistant *S. aureus* (MRSA), carbapenem-resistant *A. baumannii*, and vancomycin-resistant *E. faecium* (VRE), *E. coli* producing OXA-48 and *S. maltophilia* producing VIM.

Discussion and Conclusions

These findings suggest that EOs, particularly *Cinnamomum zeylanicum*, could represent a promising strategy to tackle antimicrobial resistance and contribute to the development of new antimicrobial agents. Further research is needed to assess clinical applicability, safety profiles, and potential synergistic effects with conventional treatments.

212 - Tracking the Evolution of Multidrug-Resistant Acinetobacter baumannii strains over six years in a University Hospital Environment

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Tracking the Evolution of Multidrug-Resistant *Acinetobacter baumannii* strains over six years in a University Hospital Environment

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Introduction
Acinetobacter baumannii has emerged globally as a significant Gram-negative aerobic cocco-bacillus and it is now recognized as a major pathogen responsible for opportunistic infections or colonization that can lead to both sepsis and multiple organ failure. Consequently, it is regarded as a high-alert human pathogen posing a serious threat to public health, requiring vigilant attention.
Material and Methods
We conducted a retrospective cohort study on patients admitted to the “L. Vanvitelli” University Hospital from 2018 to 2023 who had positive samples for *A. baumannii*. Specimens were processed and cultured according to international standards. Positive cultures were further analyzed, and species identification was performed using the MALDI-TOF BioTyper 4.1 software (Bruker Daltonics, Bremen, Germany). Antimicrobial Susceptibility Testing (AST) and Minimum Inhibitory Concentrations (MIC) were interpreted following the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).
Results
During the six-year monitoring period, we collected 10,742 blood cultures, 10,503 urine tests, and 4,693 samples including pus, drainage, and swabs. *A. baumannii* was identified in 0.8% of bloodstream samples, 0.3% of urine samples, and 1.7% of pus, drainage, and wound swabs. Among the identified strains, 89.5% were Multidrug Resistant (MDR). This period was divided into three phases: pre-pandemic (2018-2019), pandemic (2020-2021), and post-SARS-CoV-2 pandemic (2022-2023). The highest number of *A. baumannii* isolations occurred during the pandemic years, with isolations from blood cultures and urine samples increasing by 40.9%

and 62.5%, respectively. Following the implementation of an Antimicrobial Diagnostic Stewardship (ADS) program in our hospital, we observed a slight decrease in carbapenem resistance in 2020 and 2022. Additionally, the number of *A. baumannii* positive samples significantly decreased over time, with a 60.4% reduction in 2023 compared to the peak in 2020.

Discussion and Conclusion

The decreasing incidence of *A. baumannii* in our hospital may indicate the effectiveness of our ADS program, highlighting the need for continuous improvement of control measures. This study also underscores the prevalence and antibiotic resistance patterns of *A. baumannii* in the metropolitan area of our region. Constant surveillance and epidemiological studies provide clinicians with a deeper understanding of antimicrobial resistance. emphasizing the importance for specialists to be well-informed about the epidemiological data of their respective hospitals.

215 - Synergistic Antimicrobial and Antiviral Effects of Curcumin-Coated Silver Nanoparticles

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Synergistic Antimicrobial and Antiviral Effects of Curcumin-Coated Silver Nanoparticles

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Introduction:

Persistent and emerging antimicrobial resistance among microbial pathogens represents a growing global health challenge. Silver nanoparticles (AgNPs) have attracted attention for their broad-spectrum antimicrobial activity and their ability to penetrate cell membranes to target intracellular microorganisms. Moreover, natural polyphenols such as curcumin (Cur) exhibit notable antibacterial and antiviral properties. We hypothesized that functionalizing AgNPs with curcumin (AgNPs-Cur) could harness multiple, complementary mechanisms of action to yield synergistic antimicrobial and antiviral effects. This study evaluates the antimicrobial efficacy of AgNPs-Cur.

Materials and Methods:

Eco-friendly, scalable synthesis of AgNPs-Cur was carried out via a patented protocol. Antibacterial activity was determined by broth microdilution in 96-well plates, following EUCAST guidelines. Test organisms included four reference strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27953, *S. aureus* ATCC 29213, *Candida auris* DSMZ 11288) and three multidrug-resistant clinical isolates (*E. coli* ESBL, *E. coli* US, *K. pneumoniae* ESBL). Concentrations of AgNPs-Cur and free curcumin ranged from 6.25 to 200 ppm, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration preventing visible growth. Antiviral activity against SARS-CoV-2, West Nile virus (WNV), human adenovirus 1 (AdV1), and BK polyomavirus (BKPyV) was assessed by quantifying viral genomes via real-time PCR and by plaque reduction assays. Cell cytotoxicity (CC_{50}) assessments were conducted

using the MTT assay on various cell lines, based on their permissiveness to the specific virus under investigation.

Results:

AgNPs-Cur was efficient in inhibiting *E. coli*, both susceptible and resistant strains, *P. aeruginosa* and *C. auris*, with MIC ranging from 12.5 - 50 ppm, whereas *S. aureus* and *K. pneumoniae* were unaffected (MIC > 200 ppm). AgNPs-Cur was also effective against SARS-CoV-2, showing a selectivity index of 47 and a complete viral suppression at doses above 75 ppm. No antiviral activity of the AgNPs-Cur was observed when challenged against the enveloped WNV or the naked AdV1 and BKPyV.

Discussion and Conclusions:

Functionalization of silver nanoparticles with curcumin demonstrates antimicrobial efficacy specifically against most of the tested Gram-negative bacteria*, C. auris* and SARS-CoV-2, while failed to inhibit *S. aureus* and other viruses tested. This synergy suggests a promising strategy for treating multidrug-resistant infections or for coating medical devices to prevent nosocomial transmission. Further *in vivo* studies and mechanistic investigations are warranted to optimize dosing and to fully elucidate the synergistic interactions between AgNPs and curcumin.

220 - Directed evolution approach to enhance the activity of new bacteriophages targeting drug-resistant Pseudomonas aeruginosa

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Directed evolution approach to enhance the activity of new bacteriophages targeting drug-resistant *Pseudomonas aeruginosa*

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Introduction: *Pseudomonas aeruginosa* is a major opportunistic pathogen responsible for severe respiratory infections, especially in immunocompromised patients and individuals with cystic fibrosis. Its remarkable resistance to antibiotics poses a serious clinical challenge, highlighting the urgent need for alternative treatments such as bacteriophage (phage) therapy. In this study, we report the isolation, characterization, and therapeutic potential of three novel lytic *P. aeruginosa* phages.

Materials and Methods: Three environmental phages—Cisa, Nello, and Moonstruck—were isolated using clinical *P. aeruginosa* strains as hosts. Genomic characterization was performed using Illumina sequencing, followed by annotation and comparative analyses to identify genes associated with lysogeny, virulence, or resistance. The in vitro lytic activity of phages, alone and in combination with ciprofloxacin, was evaluated against a clinical *P. aeruginosa* isolate from a patient candidate for phage therapy. Furthermore, two phages (Nello and Moonstruck) underwent directed evolution through serial passage to enhance their infectivity. Phenotypic changes and genomic mutations in evolved variants were investigated.

Results: Genomic analysis classified Cisa and Nello as *Pbunavirus* members, while Moonstruck was identified as a novel *Pakpunavirus* species. All three lacked undesired genes,

supporting their therapeutic suitability. Phages Nello and Moonstruck showed lytic activity against the clinical isolate, with Moonstruck demonstrating superior efficacy, especially when combined with sub-MIC concentrations of ciprofloxacin. Directed evolution enhanced the bactericidal effect of phage Nello, with variants exhibiting prolonged bacterial suppression and mutations in tail-associated genes likely linked to improved host interaction. Moonstruck variants displayed less consistent adaptation.

Discussion and Conclusions: Our findings support the therapeutic potential of Cisa, Nello, and Moonstruck in targeting *P. aeruginosa*, particularly in drug resistant infections. The combination of phages with antibiotics and the use of phage adaptation strategies can enhance efficacy and overcome host resistance. These results expand the available phage repertoire and offer practical approaches to optimize phage therapy against multidrug-resistant pathogens.

245 - Edaravone derivatives as potential inhibitors of Quorum Sensing in Pseudomonas aeruginosa

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Edaravone derivatives as potential inhibitors of Quorum Sensing in *Pseudomonas aeruginosa*

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Introduction: The increasing prevalence of multidrug-resistant *Pseudomonas aeruginosa* strains in patients with cystic fibrosis highlights an urgent need for novel antimicrobial agents or new strategies to counteract recurrent infections. In this study we investigate the antimicrobial and antivirulence activities of quorum sensing inhibitors (QSIs). Quorum sensing (QS) is a cell-to-cell communication system mediated by the secretion of signal molecules, also known as autoinducers, which regulate gene expression, including the genes encoding virulence factors and biofilm production.

Materials and Methods: Based on the core structure of the binding between the regulatory proteins LasR/RhlR and their signal molecules (N-3-oxododecanoyl-L-homoserine lactone and N-butyryl-L-homoserine lactone respectively), we selected synthetic Edaravone derivatives (Edv) by a drug repurposing (DR)/3D-QSAR approach and molecular docking/dynamics analysis.

Edaravone was functionalized at the C4 position by introducing a fully saturated lipophilic chain (C6-C10 and C18) to increase its binding affinity to QS proteins and to act as powerful QSI.

The activity of the new QSIs was evaluated against *P. aeruginosa* reference strains. Antibacterial activity was tested by MIC determination while biofilm formation was measured using a crystal violet staining microplate assay. Reduction in pyocyanin production was evaluated by a spectrophotometric assay in presence of sub-inhibitory concentrations of the compounds. Furthermore, the expression of *lasR* and *rhlR* genes, encoding QS regulatory proteins, was analysed by RT-qPCR after exposure to the QSI.

Results: None of the compounds exhibited antimicrobial activity at concentrations ≤ 320 µg/ml; however, they reduced biofilm production at sub-MIC concentrations (80 µg/ml), particularly Edv-C10 which decreased biofilm formation by 50%. In the PAO1 control strain, treatment with Edv compounds resulted in a dose-dependent reduction in pyocyanin production, with up to a 90% decrease at ½ MIC concentrations. Gene expression analysis revealed that all three compounds reduced *lasR* and *pqsR* expression, with the Edv-C18 compound causing the greatest reduction, and being the only QSI that also reduced *rhlR* expression by 57%.

Discussion and Conclusions: These preliminary results suggest that Edaravone derivatives exhibit high affinity for the LasR and RhlR transcriptional regulator proteins, thereby interfering with the QS system and reducing the virulence of *P. aeruginosa*. Future perspectives include: (i) further optimization of Edv through drug delivery systems to enhance bacterial uptake and compound efficacy, and (ii) evaluation of antibiotic–Edv combinations against drug-resistant *P. aeruginosa* strains.

255 - Exploring the human proteome and HIV-1 genome interaction

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Exploring the human proteome and HIV-1 genome interaction

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Introduction. Human Immunodeficiency Virus type 1 (HIV-1), a member of *Retroviridae* family, targets immune cells including CD4+ T lymphocytes, macrophages, and dendritic cells. A

hallmark of its life cycle is the integration of viral DNA into host DNA, enabling lifelong persistence through latency. Since its identification, HIV-1 has infected over 80 million people and caused more than 40 million AIDS-related deaths. While antiretroviral therapy (ART) effectively suppresses viral replication, a definitive cure remains elusive due to the establishment of latent reservoirs and the virus's high genetic variability. This project aims to identify host proteins that interact with the 5' and 3' long terminal repeat (LTR) regions of the HIV-1 genome, which may uncover new therapeutic targets. To achieve this, we developed an innovative method, Long Regions of DNA Pull-down (LDP), to isolate LTR-binding proteins using biotinylated oligonucleotides corresponding to extended regions of the viral genome.

Materials and Methods. Biotinylated hybrid oligonucleotides were designed using Primer3 software to target the 5' and 3' LTRs. Each oligo contained a viral-specific sequence, and a synthetic tag absent from both human and HIV genomes to ensure specificity. The HIV-1 PNL4-3-EGFP plasmid (Vector ID: VB240829-1026wvc), containing full-length proviral DNA, was amplified in *E. coli*, purified, and used as a template for pull-down PCR generating 600 bp (5' LTR) and 300 bp (3' LTR) amplicons. These products were further amplified in a second PCR to incorporate 5'ends biotin labels. J-Lat 10.6 cells, a Jurkat-derived model harboring latent, integrated HIV-1, were cultured to obtain nuclear and cytoplasmic protein extracts. The biotinylated DNA fragments were immobilized on streptavidin-conjugated Sepharose beads and incubated with the protein extracts. After extensive washing, DNA-bound proteins were eluted and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results. The quality of the amplicons generated from both the first and second PCR reactions was assessed via 1% agarose gel electrophoresis. Nuclear protein extracts were obtained from J-Lat 10.6 cells, yielding 250 mg as determined by the Bradford assay. These extracts were subsequently used for incubation with the biotinylated amplicons generated in the second PCR. **Conclusions.** The LDP method efficiently captured specific protein-DNA interactions, enabling for the identification of both known and previously uncharacterized host factors. These findings provide a basis for future investigations into the molecular mechanisms underlying HIV-1 latency and may support the development of novel therapeutic strategies aimed at achieving a functional cure or complete eradication of the virus.

272 - Activity of Auricalaria auricula-judae extract against bacterial biofilm

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Activity of Auricularia auricula-judae extract against bacterial biofilm

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Introduction:

Auricularia auricula-judae (Bull.) Quél. (Auriculariaceae, Basidiomycetes), a lignicolous edible mushroom, is a parasite that lives on plants.

Its extrat was already tested against ATCC bacterial strains and it was showed the inhibitory effect against biofilm formation.

The purpose of this study was preferentially aimed at evaluating the inhibitory effect of extracted against biofilm formation of some clinical strains of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae*, isolated from blood samples. In case of positive action, it was also evaluated the presence and the gene overexpression correlated to biofilm production.

Materials and Methods:

The bacterial strains were inoculated in Trypticas Soy Agar media and incubated at 37°C for 24 h. The bacterial suspensions (0.5 McFarland in Broth Tryptose, BT), more precisely 100 µL of overnight grown culture, were added to a sterile 96-well flat-bottom microtiter. For controls, only the BT medium was dispensed. To evaluate biofilm production, excess bacterial suspension was removed. Subsequently, the mushroom extract (25% v/v) was re-suspended in BT, 100 µL were added to the wells, and the cultures were incubated at 37 ° C for 48h. The biofilm was colored with crystal violet dissolved in ethanol (0.5% w/v). The bound dye was released with 33% acetic acid. Finally, the optical density was measured at 540 nm. After gene presence confirmed, to evalute the expression of gene involved in the biofilm formation of strains studied, RNA was extracted from both untreated and fungus-treated bacterial strains. Then reverse transcription and Real-time PCR were performed .

Results:

The mushroom extract decreased the biofilm produced by *S. aureus* (79%) *P. aeruginosa* (8%) and *K. pneumoniae* (41%) whereas no action was highlighted by *E. coli*. The presence of genes *clfb*(*S.aureus*),*psla*(*P.aeruginosa*) and *fimh* (*K.pneumoniae*) was confirmed but was dimostrated overexpression, as highlighted in the calculation of ΔΔCt, especially for *psla* (*P.aeruginosa*) and *fimh* (*K.pneumoniae*) .

Discussion and Conclusions:

The results obtained showed a good efficacy in relation to the antibiofilm action of the *Auricularia auricula-judae* extracts against clinical strains of *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The data suggest that bacteria in contact with the fungus may likely be able to overexpress their genes as a defense mechanism against other species. The extract could be potentially applied as an antibacterial agent to control bacterial infection and to improve the pipeline for new antibiotics.

277 - In vitro evaluation of L18R peptide's antibiofilm activity against mono- and dual-species biofilms of Candida albicans and Enterococcus faecalis

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In vitro evaluation of L18R peptide's antibiofilm activity against mono- and dual-species biofilms of _Candida albicans_ and _Enterococcus faecalis_

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Introduction
The oral cavity supports biofilm formation on hard and soft tissues, where genetic and dietary factors can lead to microbial overgrowth and oral diseases. When dental structures are damaged, microorganisms may invade pulp and root canals causing endodontic infections, being *Enterococcus faecalis* common in persistent cases. Fungal species, such as *Candida albicans*, strengthen polymicrobial biofilms facilitating bacterial adhesion. Due to their complex architecture, microorganisms in biofilms are significantly more resistant to treatment than planktonic cells. In this context, antimicrobial peptides are emerging as promising agents against biofilm-related infections.
Materials and Methods
The antibiofilm activity of the previously described L18R peptide was tested on monomicrobial biofilms of *E. faecalis* (reference and vancomycin-resistant strains) and *C. albicans*, as well as on mixed yeast–bacterium biofilms. Biofilm biomass was measured by colorimetric assays and viability by CFU counts. Assays were also performed on biofilms grown on hydroxyapatite discs. Biofilm structural changes after treatment were examined using confocal laser scanning microscopy and scanning electron microscopy. Expression of genes related to biofilm formation and pathogenicity was assessed by RT-qPCR in both mono- and polymicrobial biofilms.
Results
L18R showed strong antibiofilm activity, more effective against *C. albicans* and the reference *E. faecalis* strain than the vancomycin-resistant one. Peptide activity was enhanced in polymicrobial biofilms by promoting more significant cell detachment and reduction of viability, even against less responsive bacteria. L18R affected expression of *E. faecalis* genes related to adhesion and biofilm formation and proved to destabilize biofilm structure and cause

abnormal cell detachment in both biofilm models. In *C. albicans*, transcriptional changes related to hyphal growth and adhesion appeared only in dual-species biofilm. Confocal and electron microscopy confirmed these findings, showing marked detachment of fungal and bacterial cells and increased cell death after peptide treatment.

Discussion and Conclusions

L18R exhibited antibiofilm activity by impacting both microbial viability and biofilm structure, with enhanced efficacy in the mixed-species context. The increase effect of the peptide could be related to the modulation of species-specific transcriptional responses. These findings support L18R's potential as a therapeutic agent against mixed-species biofilm-associated infections. Further studies are needed to clarify interspecies dynamics and their influence on peptide efficacy.

289 - Antiretroviral treatment modulates HERV-K and oncogenic genes to restore radiosensitivity in head and neck squamous cell carcinoma

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Antiretroviral treatment modulates HERV-K and oncogenic genes to restore radiosensitivity in head and neck squamous cell carcinoma

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Introduction: Altered expression of human endogenous retroviruses (HERVs) has been associated with the onset and progression of cancer. We previously demonstrated the simultaneous expression of HERVs and embryonic genes in aggressive stem-like cancer cells in response to microenvironmental changes. In this study, we investigated HERVs as therapeutic targets for the treatment of radiotherapy-resistant head and neck squamous cell carcinoma (HNSCC). To this end, we examined the effects of efavirenz (EFV) and zidovudine (AZT), two reverse transcriptase inhibitors, on HERVs expression, tumor aggressiveness, and cell survival in FaDu and, preliminarily, in CAL-27 cells derived from radioresistant (RR) and non-radioresistant (PAR) head and neck tumors. The combined effects of these drugs with a Rac1 inhibitor (iRAC1), a regulator of cellular processes involved in radioresistance, were assessed also during exposure to ionizing radiation**. Materials and Methods**: The FaDu-PAR and FaDu-RR cell lines were treated with AZT and EFV alone or in combination with the iRAC1 and exposed to ionizing radiation to evaluate their growth, apoptosis and the relative HERVs expression, embryonic genes, epithelial-mesenchymal transition (EMT)-related genes and nicotinamide N-methyltransferase (NNMT) by Real-Time PCR. Colony formation and wound

healing assays were also carried out in both FADU and CAL-27 cell lines (PAR and RR). **Results:** The FaDu-RR cells exhibited higher transcriptional activity than the parental cell line for HERVs, embryonic genes, NNMT, and epithelial-to-mesenchymal transition (EMT)-related genes. Treatment with antiretroviral drugs combined with iRAC1, followed by exposure to ionizing radiation, affected the transcriptional activity of most of the analyzed genes in FaDu-RR cells. We also observed a reduction in cell proliferation and the induction of apoptosis in FaDu-RR cells after the combined treatment followed by radiotherapy. Moreover, the co-treatment influenced both clonogenic activity and cell motility in the FaDu-RR and CAL-27-RR cell lines**.** **Discussion and Conclusions:** Standard cancer therapies often show limited effectiveness against certain carcinomas, particularly those that develop resistance to radiotherapy, highlighting the need for novel combinatorial treatment strategies. We demonstrated that cancer cells display elevated transcriptional levels of HERV-K. Notably, the combined use of antiretroviral drugs and iRAC1 restored the sensitivity of radioresistant cells to ionizing radiation. These findings suggest that HERV-K may contribute to the development of radioresistance and could represent a promising therapeutic target for enhancing the efficacy of radiotherapy in head and neck squamous cell carcinoma.

310 - Exploring the Antimycobacterial Potential of Podocarpusflavone A from Kielmeyera membranacea: In Vitro and In Vivo Insights

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Exploring the Antimycobacterial Potential of Podocarpusflavone A from *Kielmeyera membranacea:* In Vitro and In Vivo Insights

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Introduction The global spreading of *Mycobacterium tuberculosis* (*Mtb*) strains resistant to current antitubercular drugs poses a significant public health challenge, necessitating the urgent development of novel therapeutic agents. In chronic infectious diseases such as tuberculosis (TB), excessive inflammation contributes to severe pulmonary pathology, resulting in tissue necrosis, cavity formation, and enhanced mycobacterial dissemination and transmission. Consequently, new drug candidates exhibiting dual antimycobacterial and anti-inflammatory properties may confer substantial therapeutic advantages in aggressive forms of TB. Plant-derived compounds, particularly flavonoids, demonstrate diverse biological activities, including anti-inflammatory and antimycobacterial effects. This study focuses on podocarpusflavone A (PCFA), isolated from *Kielmeyera membranacea* (*Km*), and evaluates its efficacy against *Mtb* in extracellular, intracellular, and *in vivo* models.

Materials and Methods The PCFA was isolated from dichloromethane fraction of *Km* using Sephadex LH-20® chromatography. MIC_{50} was determined by MTT assay (*Mtb* strains) and by fluorescence detection (*M. marinum, Mm*). Cytotoxicity and bacterial growth were tested in macrophages and zebrafish model. The expression level of pro-inflammatory mediators was evaluated by RT-qPCR. C57BL/6 mice were infected and treated for 30 days with PCFA 32 µM.

Results Our findings showed that PCFA exhibited partial inhibitory effects on *Mm*, *Mtb* H37Rv and M299 (hypervirulent), with MIC50 34.83 µM, 87.38 µM and 99.61 µM, respectively. When administered in conjunction with the antitubercular agent isoniazid (INH), PCFA revealed synergistic inhibition of bacterial proliferation within THP1-derived macrophages, thus corroborating its efficacy at the intracellular level. In zebrafish caudal vein-infection model a statistically significant reduction in bacterial load was obtained using PCFA at 32 µM compared

to the infected and untreated control group. Moreover, a significant decrease in the transcriptional levels of pro-inflammatory cytokines IL-12 and TNF-α was observed, underscoring the role of PCFA in modulating the host's immune response. Finally, in the murine model, administration of PCFA at a dose of 20 mg/kg resulted in a 2.3 log10 reduction in colony-forming units (CFU) following a 30-day treatment regimen.

Discussion and Conclusions This study underscores the potential of the naturally- occurring biflavonoid PCFA, presenting evidence of its efficacy against intracellular M. tuberculosis growth, favorable safety profile, and a proposed role in modulating the host immune response. Its synergistic effect in combination with isoniazid and its evident immunomodulatory properties render PCFA a promising compound for its evaluation in host-directed adjunct therapeutic strategies.

313 - Exploring the influence of growth conditions on the high-throughput discovery of repurposed drugs for treating Pseudomonas aeruginosa lung infections in cystic fibrosis patients.

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Exploring the influence of growth conditions on the high-throughput discovery of repurposed drugs for treating *Pseudomonas aeruginosa* lung infections in cystic fibrosis patients.

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Introduction: Lung infections caused by *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients are challenging to treat due to antibiotic resistance. Finding new uses for existing drugs provides a pathway to discover novel treatments. Drug testing must align with the conditions at the site of infection. Materials and Methods: This study investigated how different growth conditions affect the activity of 3,386 compounds against *P. aeruginosa*. After 24 hours, we measured the survival of the CF strain *P. aeruginosa* RP73 under two conditions: "CF-like" (artificial CF sputum, pH 6.8, 5% CO_2) and enriched (Tryptone Soya Broth, pH 7.2). Results: Among the 3,127 non-antibiotic compounds, 13.4% were active in both conditions, but only 3.2% demonstrated similar effectiveness. Four percent were more effective in CF-like conditions, while 6.2% excelled in enriched conditions. Remarkably, 7 compounds completely killed the bacteria in CF-like conditions, whereas 12 did so in enriched conditions. Regarding the 234 antibiotics, 42.3% were active in both conditions, with only 18.4% showing similar effectiveness. Additionally, 9.4% were more effective in CF-like conditions than 14.5% in enriched conditions. Notably, 23% of antibiotics were exclusive to CF-like conditions, and 16.6% were exclusive to enriched conditions. Discussion and Conclusions: Our findings indicated that growth conditions significantly influence the activity of antibiotics and non-antibiotic drugs. Therefore, drug development and testing should reflect the actual conditions in which the bacteria exist within the body.

333 - Evaluation of the Effect of Antibiotics on the Production and Characterization of Bacterial

Extracellular Vesicles (BEVs) from Gram-Positive and Gram-Negative Bacteria

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Evaluation of the Effect of Antibiotics on the Production and Characterization of Bacterial Extracellular Vesicles (BEVs) from Gram-Positive and Gram-Negative Bacteria

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Introduction: Bacterial extracellular vesicles (BEVs) are nano-sized structures released by Gram-positive and Gram-negative bacteria, composed of lipids and proteins. BEVs play essential roles in microbial communication, stress responses, and interactions with the host. Emerging evidence suggests that antibiotics may affect not only bacterial growth but also the release and features of BEVs. This study investigates how sub-inhibitory concentrations of antibiotics influence the production and physicochemical properties of BEVs from *Staphylococcus aureus* and *Escherichia coli*.
Materials and Methods: *S. aureus* and *E. coli* were cultured in the presence and absence of selected antibiotics at ½×MIC and ¼×MIC. BEVs were isolated from culture supernatants and characterized by standard biochemical and biophysical methods, including particle size distribution, zeta potential analysis, and total protein quantification.

Results: Treatment with sub-inhibitory concentrations of antibiotics resulted in detectable differences in BEV production and physicochemical profiles. Variations were observed in vesicle yield, size distribution, surface charge, and protein content between treated and untreated conditions. Differences appeared to be both strain- and concentration-dependent, highlighting specific patterns of response in Gram-positive and Gram-negative bacteria.
Discussion and Conclusions: These findings support the hypothesis that antibiotics can modulate BEV biogenesis and composition, even at concentrations that do not affect bacterial viability. Understanding how different bacterial species adapt their vesicle production under antibiotic stress may provide new perspectives on microbial physiology and suggest potential applications for BEVs in diagnostic or therapeutic settings.

345 - Phage by phage: building a precision microbiological workflow for personalised therapy

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Phage by phage: building a precision microbiological workflow for personalised therapy

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Introduction

Phage therapy is emerging as a promising adjunct to antibiotics in treating difficult-to-manage infections. In Italy, its use is currently restricted to compassionate cases, which require formal approval by an Ethics Committee (EC). In this context, no standardized protocols for phage testing are yet available. This study aimed to develop a microbiological workflow to select the most effective phages for each patient considered for phage therapy.

Materials and Methods

Between January 1, 2021, and May 25, 2025, the Bacteriophage Laboratory at the University of Pisa received 50 phage therapy requests. For selected cases, clinical bacterial isolates were collected and screened against a curated Phage Bank and phages from international collaborators using standard plaque assays. Susceptible strains underwent *phagogram* analysis to assess phage lytic activity in liquid medium over 48 hours, helping identify early phage resistance. When susceptibility was confirmed, potential synergy with antibiotics was evaluated based on the isolate's antibiogram. Combined phage–antibiotic activity was assessed using synograms and interaction plots. Cases with favorable microbiological profiles were further reviewed for potential treatment and submitted to the EC for compassionate use approval.

Results

Of the 50 requests, 25 were accompanied by formal referrals from medical specialists and were eligible for processing. A total of 39 clinical isolates (including samples from polymicrobial infections) were received, primarily involving *Pseudomonas aeruginosa* (n=19), *Staphylococcus aureus* (n=5), *Achromobacter spp.*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Stenotrophomonas maltophilia*, and non-tuberculous mycobacteria. Initial plaque assays followed by 48-hour growth monitoring using phages alone and in combination with antibiotics (checkerboard assay) proved to be a reliable method. Phages from our collection were active against 32 of 39 isolates. For the remaining seven, ongoing efforts include targeted phage hunting and isolation. To date, 19 patients have been deemed suitable candidates for phage therapy based on clinical and microbiological evaluation. Four compassionate-use applications have been submitted to ECs, each with a personalized treatment protocol including specific antibiotic recommendations.

Discussion and Conclusions

Our early experience underscores the critical role of microbiology laboratories in enabling personalized phage therapy. Beyond access to a well-characterized phage collection, key steps include phage susceptibility testing, *phagogram* profiling, and phage–antibiotic synergy analysis. Although current regulations limit broader implementation, this work lays the groundwork for integrating phage therapy into a precision medicine framework.

346 - When one phage isn't enough: a combination approach to target Acinetobacter baumannii isolated in prosthetic joint infection

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When one phage isn't enough: a combination approach to target *Acinetobacter baumannii* isolated in prosthetic joint infection

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Introduction

The rise of difficult-to-treat infections poses a significant challenge in medical practice. Phage therapy (PT), especially when combined with antibiotics, is emerging as a promising strategy for managing infections caused by multidrug-resistant (MDR) bacteria. This study aimed to isolate and characterize novel bacteriophages active against an *Acinetobacter baumannii* strain obtained from a patient with a prosthetic joint infection (PJI) considered for PT.

Materials and Methods

Bacteriophages from the University of Pisa phage bank were screened via spot assays to identify activity against the clinical *A. baumannii* isolate. Genotypic characterization of the active phage was performed through Illumina sequencing and DNA analysis. Phage efficacy was assessed via efficiency of plating and bacterial growth kinetics. Based on the strain's antibiotic susceptibility profile, synergy testing was conducted to evaluate the combined effect of the phage and cefiderocol against both planktonic and biofilm-associated bacteria. Phage-resistant mutants were selected after 24-hour phage-bacteria co-incubation, and resistance

was confirmed by plaque assays and growth curves. A second bacteriophage specifically targeting these resistant mutants was then isolated and sequenced.

Results

Of seven phages screened, only phage L1 showed lytic activity against the A. baumannii isolate. Genomic analysis identified L1 as a lytic member of the *Friunavirus* genus, free of toxin, virulence, and antibiotic resistance genes, and thus suitable for PT. Growth kinetics revealed that all tested L1 titres effectively controlled bacterial growth for 48 hours, although resistant clones emerged. Synergistic interactions between L1 and cefiderocol were evaluated using synogram analysis and interaction plots. The combination showed an additive effect at 24 and 48 hours, preventing the emergence of resistance. Notably, L1 at 10^5 PFU/mL combined with sub-biofilm-bactericidal concentrations of cefiderocol (0.5 µg/mL) exhibited synergistic bactericidal activity against biofilm, which was not achieved with higher phage titres, possibly due to lysis from without. A second phage, ABKATE, was isolated and found active against six L1-resistant mutants. Genome analysis identified it as a novel species in the *Haddassahvirus* genus. Co-treatment with L1 and ABKATE eradicated the clinical isolate without the emergence of resistant clones.

Discussion and Conclusions

Novel bacteriophages targeting *A. baumannii* were isolated and characterized. Resistance to initial phage treatment was overcome through combination with either an antibiotic or a second phage specifically active against the mutants. The demonstrated efficacy of these phages against biofilm-associated bacteria supports their potential role in treating PJI and similar chronic infections due to MDR *A. baumannii*.

352 - Brand new results of a multicentre study on cefiderocol susceptibility in clinically relevant Pseudomonas aeruginosa isolates

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Brand new results of a multicentre study on cefiderocol susceptibility in clinically relevant *Pseudomonas aeruginosa* isolates

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Introduction: *Pseudomonas aeruginosa* is a critical pathogen, increasingly resistant to antimicrobials, especially carbapenems. Cefiderocol (FDC), a siderophore cephalosporin, shows promise against carbapenem-resistant *P. aeruginosa* (CRPa). However, its effectiveness can vary with beta-lactamase production, local resistance patterns, and challenges in antimicrobial susceptibility testing. This multicenter study aimed to evaluate FDC *in vitro* activity against *P. aeruginosa* isolates from various Italian regions.

Materials and Methods: To date, we collected 220 out of 350 expected *P. aeruginosa* isolates from respiratory, blood, and urine samples across 8 hospitals in Sicily, Calabria, and Emilia-Romagna. FDC susceptibility was assessed using disk diffusion (DD), broth microdilution in iron-depleted CAMHB (BMD), and two commercial MIC methods, ComASP and UMIC, according to EUCAST breakpoints (susceptible: MIC ≤2mg/L; DD ≥22mm). All isolates were also screened for beta-lactamase genes, including carbapenemases.

Results: All 220 isolates tested susceptible to FDC across all methods. Carbapenem resistance varied by center: Catania-Policlinico (8/50 CRPa, 6 VIM-type), Palermo-ASP (5/50 CRPa, no carbapenemases), Palermo-Policlinico (11/40 CRPa, 1 KPC), Catania-Cannizzaro (7/16 CRPa, 5 VIM, 1 NDM), Cosenza (2/16 CRPa, no carbapenemases), Cesena (3/16 CRPa, no carbapenemases), and Reggio Calabria (2/16 CRPa, no carbapenemases). Catanzaro's 16 isolates were all carbapenem-susceptible. Interestingly, some VIM-positive isolates remained carbapenem-susceptible. FDC MIC_{90} values varied slightly among methods and sites (BMD: 0.125-1mg/L; ComASP: 0.25-1mg/L), but UMIC consistently yielded higher MICs (0.5-2mg/L). FDC susceptibility didn't appear significantly linked to center-specific epidemiology. For instance, an NDM-positive isolate from Cannizzaro showed a FDC DD result in the Area of Technical Uncertainty (ATU), with BMD and ComASP indicating susceptibility, yet UMIC showed resistance (4mg/L). Although UMIC tended to give higher FDC MICs than other methods, using ComASP broth with UMIC strips improved consistency in 15 retested isolates.

Conclusions: FDC demonstrated potent *in vitro* activity against freshly isolated *P. aeruginosa* strains from Italian centers, including carbapenem-resistant strains with and without carbapenemases. This held true even in areas with high carbapenemase prevalence, like Cannizzaro Hospital. While overall susceptibility was confirmed, methodological discrepancies highlight the need for experienced clinical microbiologists and structured workflow indicating which and when use different FDC susceptibility testing methods to ensure accurate and reliable clinical results. Further NGS analyses will be performed on FDC-resistant or ATU strains.

04 4. Risposta immunitaria dell'ospite alle infezioni

12 - Insights into host innate immunity upon infection with SARS-CoV-2 VOCs

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Insights into host innate immunity upon infection with SARS-CoV-2 VOCs

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1. Introduction

Continuous surveillance and comprehensive immunological profiling are crucial for tailoring public health strategies in response to the evolving landscape of SARS-CoV-2 variants. Additionally, the interplay between host immunity and viral dynamics—which is likely to shape

the future evolution of SARS-CoV-2—remains insufficiently explored. This study investigates the differential capacity of SARS-CoV-2 variants of concern (VOCs), from the early D614G strain to post-Omicron lineages, to trigger antiviral and inflammatory responses using a human peripheral blood mononuclear cell (PBMC)-based in vitro model.

2. Materials and Methods

To assess immune activation by different VOCs, human PBMCs were stimulated in vitro and analyzed 24 hours post-stimulation. Real-time RT-PCR was used to quantify type I interferon (IFN) production and IFN-induced Mx1 gene transcription. Additionally, a multiplex cytokine-bead array was employed to evaluate the release of inflammatory cytokines and chemokines, including interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), CXCL-8 (IL-8), CCL-2 (MCP-1), and CCL-5 (RANTES).

3. Results

Distinct antiviral gene expression signatures and cytokine/chemokine profiles were observed across different SARS-CoV-2 VOCs. The Beta variant exhibited a notably lower ability to induce cytokine and chemokine production compared to Omicron BA.1, both in PBMCs and in isolated $CD14^+$ monocytes. These immunological disparities aligned with differences in viral subgenomic RNA (sgRNA) profiles. Although PBMCs do not support productive SARS-CoV-2 replication, the presence of sgRNAs suggests their involvement in modulating immune signaling pathways and cytokine secretion. SARS-CoV-2 RNA was detectable in all examined immune cell types, with a predominant presence in $CD14^+$ monocytes. This led to further characterization of monocyte responses to infection with Beta and Omicron BA.1 variants. Notably, monocytes stimulated with the Beta variant exhibited higher mortality, as shown by increased apoptosis, elevated Annexin-V expression, and higher lactate dehydrogenase release compared to BA.1-treated cells. These effects were associated with greater viral release early post-infection, despite similar levels of viral entry by both variants.

4. Discussion and Conclusions

This study reveals that SARS-CoV-2 variants of concern differentially modulate immune responses, especially in monocytes, by affecting apoptotic, inflammatory, and antiviral pathways. These insights highlight how variant-specific immune interactions may influence disease severity and viral evolution, guiding the development of targeted therapies and public health strategies.

25 - Caspase-8 inhibition activates a neutrophil-centric inflammatory pathway that enhances host defense against antibiotic-resistant bacteria

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Caspase-8 inhibition activates a neutrophil-centric inflammatory pathway that enhances host defense against antibiotic-resistant bacteria

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Introduction: The rising prevalence of infections caused by antimicrobial-resistant bacteria underscores the urgent need for effective host-directed therapies. Traditional strategies aimed at boosting immune responses have often been limited by the risk of exacerbating systemic inflammation, leading to cytokine storms and septic shock. In this study, we identified caspase-8 as a key regulator of inflammation, modulating tonic TNF-α-driven transcriptional changes in neutrophils during homeostasis.

Materials and Methods: Therapeutic efficacy of the caspase-8 inhibitor z-IETD-fmk was evaluated using the cecal ligation and puncture (CLP) model, a widely accepted murine model that recapitulates key features of human peritonitis and sepsis. Host responses were assessed by quantifying neutrophil recruitment and cytokine levels in body fluids and organ homogenates.

Results: Inhibition of caspase-8 initiates a neutrophil-centered inflammatory program that enhances bacterial clearance. This effect is mediated through a cell death–independent pathway involving autocrine TNF-α signaling, RIPK1/3 phosphorylation, and NF-κB- and MAPK-dependent activation of proinflammatory and chemotactic cytokine genes. Notably, this inflammatory cascade occurs independently of canonical inflammasome components such as NLRP3 and ASC. Instead, IL-1β release requires gasdermin D (GSDMD) processing, revealing a novel inflammasome-independent mechanism of cytokine secretion.

Discussion and Conclusions: Caspase-8 inhibition drives a protective, neutrophil-driven inflammatory response that enhances bacterial clearance and improves survival in a lethal sepsis model. These findings highlight caspase-8 as a promising therapeutic target for host-directed treatment strategies against antibiotic-resistant infections.

32 - Activation of endosomal Toll-like Receptors enhances host defense against Klebsiella pneumoniae

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Activation of endosomal Toll-like Receptors enhances host defense against *Klebsiella pneumoniae*

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Introduction: The rising incidence of difficult-to-treat infections caused by hypervirulent *Klebsiella pneumoniae* underscores the need for effective host directed therapies against this pathogen. Effective immune recognition of *K. pneumoniae* depends on pattern recognition receptors (PRRs), particularly Toll-like receptors (TLRs), expressed by phagocytes. However, the relative contribution of individual TLRs to host defense against *K. pneumoniae* and other Gram-negative bacteria remains poorly understood. This study focuses on the role of nucleic

acid-sensing endosomal TLRs in orchestrating proinflammatory and chemotactic immune responses.

Materials and Methods: Genetically engineered TLR-defective mice underwent experimental *K. pneumoniae* pneumonia and were observed for the presence of clinical signs. Animals showing signs of irreversible disease were humanely euthanized. Bacterial loads were quantified via colony-forming unit (CFU) assays from organ homogenates. Cytokine and chemokine production in macrophages and neutrophils derived from mutant mice was evaluated after stimulation with whole bacteria or *K. pneumoniae*-derived nucleic acids.

Results: Mice lacking functional endosomal TLR signaling (or "3d" mice) showed significantly increased susceptibility to *K. pneumoniae*, characterized by impaired bacterial clearance and enhanced systemic dissemination. These mice exhibited defective neutrophil recruitment and attenuated cytokine responses during infection. The altered immune response correlated with diminished release of CXCL2, CXCL1, IL-1β, IFN-β, and TNF-α by macrophages polarized with either M-CSF or GM-CSF. Alveolar macrophages from 3d mice displayed immune mediator expression patterns similar to those in TLR2- and TLR4-deficient animals. Additionally, *K. pneumoniae* nucleic acids, particularly RNA strongly induced proinflammatory cytokines in macrophages, with TLR7, TLR9, and TLR13 playing prominent roles in driving neutrophil activation, ROS production, and bacterial killing.

Discussion and Conclusions: These findings reveal that functional endosomal TLR signaling is essential for effective immune responses against *K. pneumoniae*, particularly through macrophage-derived cytokine production and neutrophil-mediated bacterial clearance. The results underscore the importance of bacterial RNA as a key pathogen-associated molecular pattern (PAMP) recognized by endosomal TLRs TLR7 and 13 during infection. This study provides new insights into the innate immune mechanisms involved in combating *K. pneumoniae* and paves the way to strategies aimed at targeting endosomal TLRs to enhance host defense against multidrug-resistant bacterial pathogens.

36 - Analysis of anti-ZIKV innate immune responses using an immunocompetent mouse infection model

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Analysis of anti-ZIKV innate immune responses using an immunocompetent mouse infection model

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Introduction: Zika virus (ZIKV) is an arthropod-borne flavivirus that can cause widespread epidemics associated with complications such as encephalopathy, Guillain-Barrè syndrome and microcephaly. The emergence of ZIKV and the rapid spread of its vector has promoted the development of models to understand several aspects of ZIKV pathogenesis, including innate immune responses. The final aim of our studies is to identify alternative therapeutic strategies by investigating a recently discovered ZBP1/RIPK3 defense pathway and evaluate its usability as a drug target. Since ZIKV specifically inhibits the activation of human Signal Transducer and Activator of Transcription 2 (STAT2), but not its murine counterpart, immunocompetent mice are resistant to infection. As of today, the most used models involve mice lacking components of the type I interferon (IFN-I) signaling pathway, which are not ideal for investigating innate immune responses. To avoid this problem, we havé set up an infection model using immunocompetent mice.

Materials and methods: Newborn mice were used to create a model of persistent ZIKV infection in the brain that recapitulates some features of ZIKV infection in the human fetus. In this model, immunocompetent C57BL/6 inbred mice are inoculated subcutaneously with 3.4×10^5 plaque-forming units (PFU) of the Brazil/2016/INMI1 (009V-00880) ZIKV strain at 24-48 hours after birth. Puppies showing severe disease are euthanized and organs such as brain and spleen are collected for viral quantification through qPCR and enumeration of plaque-forming units.

Results: The subcutaneous administration of ZIKV to neonatal mice resulted in reduced weight gain and neurological symptoms including hind limb paralysis, tremors, decreased tail tone and impaired rightening reflex. Symptoms typically emerge around day 10 post-infection and

by day 20 the mortality rate reaches 100%. Notably, severity is age-dependent since older mice exhibit decreased susceptibility.

Discussion and Conclusions: These findings indicate that neonatal C57BL/6 mice provide a reproducible model of neurological ZIKV infection. This model is being currently used to evaluate the susceptibility of 4 different types of transgenic mice on a C57BL/6 background with specific defects in the ZBP1 pathway. Moreover, a CD1 outbred mouse model of ZIKV infection has also been set up to provide additional means of assessing the ability of antiviral drugs and vaccines.

38 - AIEC-derived LPS and Outer Membrane Vesicles differently drive the dysregulated immunological pathways linked to Crohn's Disease.

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AIEC-derived LPS and Outer Membrane Vesicles differently drive the dysregulated immunological pathways linked to Crohn's Disease.

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Introduction: CD is a chronic inflammatory bowel disease linked to a mucosal enrichment of the adherent-invasive *E. coli* (AIEC) pathotype and a strong expansion of pathogenic Th17 (pTh17) cells. We previously proved that the deletion of the LPS-core heptose kinase RfaP, significantly impairs the IL-23 secretion by dendritic cells (DCs) and hampers pTh17 cell generation. Given the pivotal role of LPS and Outer Membrane Vesicles (OMVs) in triggering several proinflammatory processes, we aimed to deeply characterize the AIEC virulence determinants specifically implicated in the detrimental activation of the immune system in CD.
Materials and Methods: LPS and OMVs were purified from AIEC-LF82 strain, from its isogenic deletion mutants in the Type 1 fimbria adhesin (Δ*fimH),* and in specific genes of the LPS core biosynthesis operon (e.g. Δ*rfaP*), respectively involved in the invasion of intestinal epithelial cells (IECs), persistence within DCs, and pTh17 cell generation. Intracellular bacterial load and host inflammatory responses, upon infection with live bacteria or purified LPS/OMVs, were quantified in HT29 IECs, human macrophages (MDMs) and DCs.

Results: The double mutant Δ*rfaP/*Δ*fimH* showed an impaired ability to invade IECs and a complete loss of intracellular persistence. Notably, IL-8 and CCL-20 secretion resulted strongly diminished in response to Δ*rfaP/*Δ*fimH* double mutant*,* compared to single LPS mutants or Δ*fimH*. Similarly, all LPS mutants, Δ*rfaP/*Δ*fimH,* but not Δ*fimH*, displayed defective intracellular persistence within MDMs and DCs. Of note, none of tested mutants displayed a reduced TNFa secretion by MDMs, whereas all the LPS mutants and Δ*rfaP/*Δ*fimH* strongly impaired the IL-23/IL-1b secretion by DCs. Interestingly, LPS induced a comparable inflammatory response to that triggered by live bacterial strains exclusively in IECs, while it induced only weak cytokines release in MDMs and DCs. In contrast, OMV promoted a selective higher inflammatory response in DCs compared to LPS. Intriguingly, LPS mutants and Δ*rfaP/*Δ*fimH* secreted significantly higher OMV compared to LF82 but induced a lower proinflammatory response.

Discussion and Conclusions:

The AIEC-host cells interplay involves different virulence determinants, with LPS and Type 1 fimbriae promoting intracellular persistence and pro-inflammatory response in IECs and MDMs, while OMVs selectively drives polarizing cytokine secretion by DCs. Particularly, simultaneous targeting of RfaP and FimH in AIEC showed an impaired persistence within host cells and hamper the immunological pathways highly dysregulated in CD. In conclusion, RfaP represent a paramount bacterial target that, together with FimH inhibitors, could be pivotal for the development of novel and more effective therapeutic strategies for CD.

39 - P. aeruginosa infection promotes the skewing of protective Th17 cells into pathogenic Th1/17 cells with a distinct transcriptomic profile and TCR clonotype.

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P. aeruginosa **infection promotes the skewing of protective Th17 cells into pathogenic Th1/17 cells with a distinct transcriptomic profile and TCR clonotype.**

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Introduction: Cystic Fibrosis (CF) is a genetic disease characterized by a chronic non-resolving inflammatory response to bacterial lung infections. In this context, *P. aeruginosa* (Pa) is one of the most prominent pathogens in CF, promoting dysregulated immune system activation and a rapid decline in pulmonary function. In addition to the host-damaging inflammatory roles of neutrophils and macrophages, IL-17/IFNg co-producing Th1/17 cells are enriched in the lungs of CF murine models in response to Pa, suggesting a pivotal pathogenic role for these cells. However, the link between Pa infection and the generation of human pulmonary Th1/17 cells, as well as the Pa-virulence determinants directly involved in pathogenic Th1/17 activation, remains elusive.

Materials and methods: Peripheral and pulmonary T cell subsets in CF patients were quantified by flow cytometry. The pathogenicity of Th1/17 cell subsets was evaluated using a pulmonary epithelial ALI model. Transcriptomic profiles and TCR clonotypes of peripheral and pulmonary Th1/17 subsets were analyzed by RNA-Seq. The ability of Pa to directly promote Th1/17 cell transdifferentiation was evaluated by an in vitro antigen-dependent functional assay.

Results: Among lung-infiltrating T cells, $CCR5^{+}$Th1/17 cells were significantly and selectively enriched in chronically Pa-infected lungs of CF patients. In the primary human ALI epithelial cell model, we observed extensive damage to epithelial barrier integrity and the release of high levels of proinflammatory cytokines exclusively in the presence of activated Th1/17 subsets. Comparative analysis of transcriptomic profiles revealed distinct molecular signatures among CF pulmonary Th1/17 subsets, and the expansion of specific TCR clonotypes in CF lung-infiltrating $CCR5^{+}$Th1/17 cells. Clinical Pa strains significantly persisted within human dendritic cells triggering the release of high levels of IL-1b and IL-23 linked to Th1/17 cell differentiation. Interestingly, among different virulence phenotype analyzed, only biofilm production significantly correlated with IL-1b secretion and intracellular persistence of early, but not late, Pa strains. Finally, Pa isolates triggered the skewing of pathogenic Th1/17 cells exclusively from protective Th17 cells and not from Th1, with no significant differences between early and late isolates.

Discussion and conclusions: Pa chronically persists within DCs, promoting the massive generation of pathogenic Th1/17 cells, which in turn induce tissue damage and contribute to lung function decline. Deciphering the molecular mechanisms underlying Pa-dependent Th1/17 cell generation could lead to the identification of crucial bacterial and immunological targets for the development of more effective therapeutic strategies in CF.

46 - S. pneumoniae clearance by human spleen macrophages is complemented through apoptosis activation

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S. pneumoniae clearance by human spleen macrophages is complemented through apoptosis activation

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Introduction

The bacterium *Streptococcus pneumoniae* is one of the major causes of serious diseases and mortality, causing pneumonia, meningitis, and sepsis. In particular, while *S. pneumoniae* is known to be targeted by phagocyte-mediated clearance in the spleen, the role of splenic macrophages has not been fully elucidated.

Materials and Methods

To investigate how splenic macrophages respond to *S. pneumoniae*, we propose an innovative *ex vivo* human spleen perfusion model. Tissue biopsies were analyzed using immunohistochemistry to detect markers associated with macrophage activation, including activated caspase-3 (cas3), its target M30 and the lysosome-associated membrane protein 1 (lamp1). These markers provide critical insights into macrophages and tissue response.

Results

Our findings demonstrate that the human spleen plays a crucial role in capturing and removing *S. pneumoniae*. Within the first hour of perfusion, 90% of the bacteria was cleared, highlighting the spleen efficiency in capsulated bacteria removal. Immunohistochemistry analysis revealed a ten-fold increase in cleaved-cas3 levels within macrophages at one hour post-infection, suggesting that macrophage apoptosis may play a role in the bacterial clearance. Over time, both the number of bacteria and the levels of activated-cas3 decreased, indicating a dynamic correlation between bacterial killing and macrophage cell-death. Moreover, this process was followed by the increase in cleaved-M30 levels at later time points. This reflects prolonged cas3 activation, suggesting sustained apoptotic activity in macrophages during the immune response to pneumococci. Despite these changes, the percentage of lamp1-positive macrophages did not increase significantly, suggesting that lysosomal activity might not be the only mechanism for pneumococcal killing. Our results indicate that macrophage cas3 mediated cell-death may complement other immune responses to effectively control *S. pneumoniae*.

Finally, we observed that CD169+ macrophages harbor 10 times more cas3 than CD163+, suggesting that these cells may serve as a reservoir for the initial stages of invasive pneumococcal infections. In addition, out of 10^{10} macrophages, 10^{8} are positive for activated-cas3. Considering an infection dose of 10^{8} and assuming that each macrophage takes one bacterium, each cell that internalizes a bacterium undergoes apoptosis.

Discussion and Conclusions

This study provides evidence that splenic macrophages play a critical role in the clearance of pneumococci, involving cas3 mediated cell-death. These findings could contribute to the development of more effective treatments to prevent the transition of *S. pneumoniae* from localized infections to systemic disease, and enhance our understanding of the spleen-mediated immunity.

54 - Role of inflammatory mediators, endothelial dysfunctions and TTV levels in the pathogenesis of respiratory diseases

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Role of inflammatory mediators, endothelial dysfunctions and TTV levels in the pathogenesis of respiratory diseases

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Introduction: Aberrant immune responses and cytokine production have been implicated in the pathogenesis of viral infections. Respiratory virus (RV) infections have been strongly associated with multi-systemic inflammatory disease, overproduction of a wide range of proinflammatory mediators. (characterized by cytokine release syndrome), thromboembolic phenomena and microcirculatory dysfunction, leading to severe to critical illness in patients. Main objective of this study is to investigate the role of RV infections in severe RV-related pathogenesis, focusing on the mechanisms that trigger systemic inflammation, thrombotic events and TTV levels.

Materials and Methods: The levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, interferon (IFN)-γ, IFN-α, IFN-β, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion protein 1 (VCAM-1) were measured in 256 serum samples from patients positive for various RV (116 (45.3%) influenza (FLU), 52 (20.3%) respiratory syncytial virus (RSV), 51(19.9%) rhinovirus (HRV)) and 22 samples from control group by an automated immunoassay (Protein Simle, Biotechne). TTV levels were quantified by in house RT-PCR in serum samples and expressed in TTV log/ml. Kruskal-Wallis test, followed by Dunn's multiple comparison test, was used for group comparison. Correlations were determined using Spearman's correlation.

Results: IL-6 expression was significantly higher in severe FLU patients than in non-severe FLU patients ($p \leq 0.05$). IFN- α expression is statistically significantly higher in FLU-positive compared to HRV-positive patients. IL-8 is significantly higher in FLU patients compared to RSV patients. VCAM-1 is significantly higher in FLU than RSV and HRV patients. TTV levels are greater in non-severe FLU-positive patients respect to severe patients. TTV log/ml showed positive correlation with IL-6 in the 0-1 year old age group while, in the 81-94 age group, showed a negative correlation with IFN-α, TNF-α and ICAM-1. In the age group 19-59, TTV levels showed a negative correlation with ICAM-1.

Discussion and Conclusions: Additional information on the relationship between respiratory viruses and inflammatory mediators was provided by the study results. IL-6 is more expressed in FLU severe patients, recently results published in the literature suggest that inhibition of IL-6 release may be a new therapeutic strategy for emerging fatal complications mediated by the "cytokine storm". HRV stimulated IFN-α production indicating a hyperactive immune response but not in other RVs. A significant increase in IL-8 release in serum samples is associated with respiratory disease due to RSV infection. Serum VCAM-1 levels were significantly higher in FLU-positive patients indicating a possible excessive inflammation, which can damage tissues and worsen respiratory symptoms. The levels of TTV are statistically higher in non severe FLU-positive individuals, an indication of reduced function of the immune system. In general, more research is needed to investigate the correlation with clinical outcomes.

56 - Goblet cell breakdown: Transcriptomics reveals Acinetobacter baumannii early and robust inflammatory response in differentiated human bronchial epithelial cells

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Goblet cell breakdown: Transcriptomics reveals *Acinetobacter baumannii* early and robust inflammatory response in differentiated human bronchial epithelial cells

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Introduction. The airway epithelium represents the first line of defense of the lungs, functioning both as a physical barrier as well as an active immune modulator. However, in the last years, pneumonia caused by the opportunistic pathogen *Acinetobacter baumannii* have become difficult to treat due to the increase of the number of extensively drug resistant strains. Materials and Methods. In this study, we report for the first time the use of an *ex vivo* air-liquid interface (ALI) model of differentiated human bronchial epithelial cells to unravel the early response to *A. baumannii* infection. Epithelial integrity, tissue architecture, and goblet cell function were assessed through FITC-dextran permeability assays, hematoxylin and eosin

staining, and indirect immunofluorescence. Transcriptomic profiling was performed to characterize host gene expression changes. Results. Initial tissue damage began as early as at 4 hours post-infection (hpi); at 24 hpi, goblet cell hypertrophy, reduced mucin secretion, and compromised epithelial integrity were highly evident. Transcriptomic data at 4 hpi revealed 668 differentially expressed genes (441 upregulated, 227 downregulated), mainly involved in a strong pro-inflammatory response and characterized by IL-8/CCL20-driven neutrophil recruitment and type 2 cytokine activation (IL-4, IL-13). Noteworthy, genes related to cytoskeletal organization, adhesion, and extracellular matrix remodeling were significantly altered, suggesting a bacterial mechanism to enhanced tissue dissemination. The PI3K-Akt survival pathway was inhibited, with downregulation of *PIK3R1* and *PIK3R2* genes, implying the induction of apoptosis/cell death and epithelial damage. Our findings are in agreement with previous *in vivo* studies, further strengthening the value of our ALI model in mimicking the early infection response of bronchial cells to *A. baumannii* infection. Discussion and Conclusions. Our data highlight the early molecular mechanisms underlying *A. baumannii* pathogenesis and open new avenues for future investigations for therapeutic interventions.

73 - The European Interest on HPV Vaccination in the Era of Innovative Vaccines: Real-Time Surveillance Using Google Trends

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The European Interest on HPV Vaccination in the Era of Innovative Vaccines: Real-Time Surveillance Using Google Trends

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Introduction: Over the past two decades, the development of prophylactic Human Papillomavirus (HPV) vaccines represented one of the most significant advancements in cancer prevention of cervical, vulva, vagina, mouth/throat, penis and anus. To date, 38 out of 48 European countries have integrated preventive HPV vaccination into their National Immunization Programs. Despite its proven safety, effectiveness, and availability, the anti-HPV vaccine uptake among the targeted population remains suboptimal, with coverage rates of 35% in females and 20% in males across European regions. This observational study aimed to evaluate interest related to the HPV vaccination topic and determine the year-over-year changes in searches as well as differences across European areas. **Materials and Methods:**

Google Trends was queried to explore online searches on “HPV vaccine”, using relative search volumes (RSVs), from January 2007 to December 2024. A regression model analysed differences in searches each year. Statistically significant changes over time were set up to ≤0.05. **Results:** The year-wise online search volume for the HPV vaccine showed significant variations across Europe. Germany showed a higher increase in interest per year than the European average ($p < 0.001$), followed by Slovakia ($p = 0.003$), Romania ($p = 0.004$), Italy ($p = 0.004$), and Bulgaria ($p = 0.012$). France showed moderate interest, while Belgium and the Netherlands had a lower year-wise online search volume rate. Denmark ($p < 0.001$) and Iceland ($p = 0.016$) exhibited significantly lower levels of interest compared to the European average, possibly due to their already high HPV vaccine coverage rates of 79% and 84%, respectively, which may reduce the need for public information-seeking behaviour. **Discussion and Conclusions:** Interestingly, Eastern and Southern European countries showed a significant increase in interest per year compared to other European areas, which may reflect increasing public awareness in these regions; while in Western Europe, such as France, Belgium and the Netherlands exhibited lower levels of interest, potentially highlight the need for tailored public health strategies. These preliminary results underscore the need for targeted public health campaigns and educational initiatives to address regional disparities, promote vaccine uptake, and emphasize the importance of HPV vaccination in cancer prevention strategies.

78 - Immune modulatory effects of Pseudomonas aeruginosa microvesicles

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Immune modulatory effects of *Pseudomonas aeruginosa* microvesicles.

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Introduction: *Pseudomonas aeruginosa*, a Gram-negative bacterium, is an opportunistic pathogen producing several virulence factors in response to quorum sensing (QS) system activation. Indeed, *P.*
aeruginosa uses QS-related autoinducing molecules such as homoserine lactones (i.e., 3OC12-HSL) or 2-heptyl-3-hydroxy-4 (1H)-quinolone (PQS) to modulate host immunity. Bacterial membrane vesicles (BMVs) are reported to carry factors involved in host-pathogen interactions, thus modulating infection outcomes. However, protocols for isolating and characterizing BMVs and insights into the BMVs-related mechanisms are still lacking.
Material and methods. BMVs were isolated from cultures of *P. aeruginosa* standard strain (PAO1) and QS-deficient mutant (deltaQSPAO1) through sequential centrifugation, ultracentrifugation, and size-exclusion chromatography. BMVs quantity and integrity were assessed via Nanoparticle Tracking Analysis (NTA) and flow cytometry. The presence of 3OC12-HSL and PQS was assessed in BMVs using biosensors. To investigate immune-modulatory effects, BMVs were incubated with primary human macrophages previously polarized to pro-inflammatory (M1) or anti-inflammatory (M2) phenotype; cytokine levels were determined in the conditioned media.

Results. NTA characterized the purified BMVs yielding a concentration of 10^{10} particles/ml with an
average diameter of 163 ± 1.2 nm. Biosensor assays confirmed the presence of QS molecules 3OC12-HSL and PQS associated with BMVs, showing dose-dependent responses. Notably, BMVs collected from PAO1 cultured with sub-MIC of ciprofloxacin exhibited increased 3OC12-HSL and PQS content. Macrophage stimulation demonstrated that BMVs from PAO1 grown with ciprofloxacin significantly reduced pro-inflammatory cytokine levels in lipopolysaccharide activated M1 macrophages. Conversely, BMVs from PAO1 cultured with 3OC12-HSL stimulated M2 macrophages to produce anti-inflammatory factors. BMVs obtained from deltaQSPAO1 showed no significant immune-regulatory effects.
Discussion and Conclusions. This study reveals the critical role of *P. aeruginosa* derived BMVs in mediating host-pathogen interaction. The enhanced presence of QS molecules following ciprofloxacin treatment suggests that BMVs may serve as key signals in modulating host immune activity during antibiotic therapeutic interventions. By reducing pro-inflammatory cytokines in M1 macrophages and promoting anti-inflammatory responses in M2 macrophages, BMVs demonstrate their potential as biomarkers in infections.

80 - Linking microbiome dynamics to disease susceptibility and severity in a mouse model of invasive non-typhoidal Salmonella infection

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Linking microbiome dynamics to disease susceptibility and severity in a mouse model of invasive non-typhoidal *Salmonella* infection

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Introduction. *Salmonella enterica* serovar Typhimurium (STm) is a major cause of foodborne illness globally. While it typically induces self-limiting gastroenteritis in immunocompetent individuals, it can cause severe systemic infections in immunocompromised hosts. Improved animal models that mimic human infections are crucial to study invasive non-typhoidal *Salmonella* (iNTS) pathogenesis. In this study, a murine model of STm enteric infection was established and characterised. This model induces a typhoid-like systemic disease in susceptible C57BL/6 mice without streptomycin pre-treatment, preserving the normal gut microbiota. We sought to correlate susceptibility to infection and disease progression with several clinical parameters, as well as gut microbiome composition.
Materials and Methods. C57BL/6 mice were intragastrically infected with 10^8 CFU/mouse of the clinical isolate D23580 without antibiotic pre-treatment. At days 0, 1, 3, and 7 post-infection (p.i.), faecal samples, cecum, colon, blood, liver and spleen were collected to assess alpha- and beta-diversity in gut microbiome, bacterial loads, histopathology and cytokine production.
An in-house bioinformatic pipeline was developed to process raw reads obtained by full-length 16S rRNA gene sequencing (Nanopore) into taxonomic abundance tables for alpha- and beta-

diversity analysis. An integrative study was then performed to correlate microbiome data with disease progression parameters to identify host factors associated with susceptibility to STm infection.

Results. Microbiome composition was correlated, by a multi-parametric approach, with clinical scoring as weight loss, bacterial burden in both intestinal and systemic compartments, histopathology and cytokine profiling. Microbial shifts in alpha- and beta-diversity emerged early and reflected infection progression and severity over time. Gastrointestinal colonization by STm was detected from day 1, up to day 7 p.i. with increasing systemic dissemination and clinical sign severity over time. Clinical manifestations were supported by histopathological assessment of leukocyte infiltration, tissue damage and vascular thrombosis in intestinal and systemic compartments, paralleling gut microbiome and inflammatory alterations.

Discussion and Conclusions. This iNTS murine model, which mimics key aspects of human typhoid-like systemic disease, provides a valuable framework to investigate the relationship between gut microbiome dynamics, susceptibility to STm infection, and disease progression. This study highlights the value of an integrative approach to host–pathogen–microbiota interactions, facilitates the development of predictive biomarkers for disease outcomes, and offers a robust tool for testing novel therapeutic and prophylactic strategies.

90 - Antimicrobial and Immunomodulatory Potential of Fractionated Cell-Free Supernatants from Lacticaseibacillus rhamnosus Against Pseudomonas aeruginosa

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Antimicrobial and Immunomodulatory Potential of Fractionated Cell-Free Supernatants from *Lacticaseibacillus rhamnosus* Against *Pseudomonas aeruginosa*

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Introduction: The rise of multidrug-resistant pathogens and the loss of antibiotic efficacy have increased interest in probiotics and their metabolic products as alternative antimicrobial strategies. Among these, cell-free supernatants derived from lactic acid bacteria are emerging as promising candidates. Moreover, cell-free supernatants have also shown immunomodulatory effects, suggesting they could be beneficial in infectious settings associated with chronic inflammation, such as the lungs of cystic fibrosis patients. In this study, we aimed to identify the active fraction(s) in cell-free supernatants from *Lacticaseibacillus rhamnosus* (CFS) and to characterise their immunomodulatory properties and protein composition. **Methods:** A clinical *Pseudomonas aeruginosa* isolate was used. CFS fractions were obtained by size-exclusion filtration with a 3 kDa cut-off. Antibacterial and antibiofilm activity of whole and fractionated CFS were assessed against planktonic and biofilm-associated *P. aeruginosa* by colony-forming unit enumeration, crystal violet staining, and confocal microscopy. Label-free qualitative proteomics was carried out via HPLC–MS/MS to characterise the CFS protein content. Additionally, the immunomodulatory effects of the CFS were evaluated on human peripheral blood mononuclear cells (PBMC) stimulated with *P. aeruginosa* lipopolysaccharide (LPS) or *P. aeruginosa* biofilm cells. **Results:** Antibacterial and antibiofilm effects were mainly, though not exclusively, attributed to the low molecular weight CFS fraction (≤ 3 kDa), which contained most of the lactic acid, suggesting its central role in the antimicrobial effects. The > 3 kDa fraction, when tested alone, was almost inactive but exhibited a synergistic antibacterial effect when reconstituted with the ≤ 3 kDa fraction. Proteomic analysis of CFS identified the presence of cell wall hydrolases, indicating that these enzymes may contribute to the antibacterial activity observed in the reconstituted fractions. After 6 h stimulation of PBMC with LPS or biofilm-derived *P. aeruginosa*, a marked anti-

inflammatory effect was observed in both the unfractionated CFS and the ≤ 3 kDa fraction at non-toxic concentrations. In contrast, the > 3 kDa fraction induced the production of IL-6, TNF-α and, to a lesser extent, IL-10. **Discussion and conclusion:** The results suggest that probiotic metabolites, with their antimicrobial and anti-inflammatory properties, could offer a promising strategy for the prevention and/or treatment of chronic infections with inflammatory responses, such as those caused by *P. aeruginosa*.

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98 - Immunomodulatory properties of heat-inactivated biomasses by selected bacterial and fungal species included in the Qualified Presumption of Safety list of the European Food Safety Authority

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Immunomodulatory properties of heat-inactivated biomasses by selected bacterial and fungal species included in the Qualified Presumption of Safety list of the European Food Safety Authority

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Introduction: The use of microbial lysates has been described to prevent recurrent respiratory infections, to avoid the flare-up of respiratory chronic infections and as a defence against urinary infections by modulating host responses with partially understood mechanisms. Recently, we have demonstrated that a *Cutibacterium acnes* bacterial lysate (BL) enhances cellular responses against *Candida albicans*, *Escherichia coli*, and *Gardnerella vaginalis* in an *in vitro* model of vaginal infection, through mechanisms associated with trained immunity. The present work aims to identify new microbial lysates with immunomodulatory capacity comparable to or better than BL, to be tested in an *in vitro* model of *E. coli*-induced urinary tract and gastrointestinal infection.
Materials and Methods: Ten microorganisms (6 fungi, 3 Gram-positive bacteria and 1 Gram-negative bacterium), included in the Qualified Presumption of Safety (QPS) list published by the European Food Safety Authority (EFSA), were selected. Microorganisms were cultured in broth and incubated at 30 °C for at least 48 h. Microbial growth was monitored by measuring optical density (OD) and colony-forming units (CFU). Subsequently, the cultures were heat-inactivated at 75 °C for 3 h. The resulting heat-inactivated microbial biomasses (Hi-MBs) were harvested by centrifugation and dried overnight using a speed-vacuum system. The murine macrophage cell line J774A.1 was then primed with the obtained Hi-MBs for 24 h at 37 °C and 5% CO_2. Then the phagocytic activity *versus E. coli* fluorescent bioparticles, was assessed after 30 min or 2 h, using the Vybrant™ Phagocytosis Assay Kit according to the manufacturer's instructions.
Results: In a first series of experiments, macrophages primed with Hi-MB obtained by the yeast *Hanseniaspora uvarum* (*H. uvarum*) and the Gram negative bacterium *Xanthomonas campestris* (*X. campestris*) exhibited a significant increase in phagocytic activity with respect to un-primed controls. Such activity was comparable to the phagocytic activity observed in cells primed with either BL or lipopolysaccharide (LPS), indicating that *H. uvarum* and *X. campestris* Hi-MBs could be effective in triggering macrophages activation.
Discussion and Conclusion: Our results suggest that *H. uvarum* and *X. campestris* Hi-MBs could effectively train innate immune cells, thus supporting the possibility to be employed for the development of new microbial lysates with immunomodulatory properties. Future studies will evaluate whether these lysates can also enhance mitochondrial activity and microbicidal efficacy and if they are able to improve cellular responses to *E. coli* in an *in vitro* model of urothelial and intestinal infection.

121 - Vitamin D3 contribution to systemic and local control of Salmonella Typhimurium infection in an in vivo mouse model

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Vitamin D3 contribution to systemic and local control of *Salmonella* Typhimurium infection in an *in vivo* mouse model

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Introduction. *Salmonella enterica* serovar Typhimurium (STM) represents a major global public health threat, causing a wide range of illnesses, ranging from mild, self-limiting to severe gastroenteritis. Recent studies have demonstrated that Vitamin D3 (VitD3), particularly its active metabolite 1,25-dihydroxyvitamin D3, exerts antimicrobial effects by modulating innate and adaptive immunity in murine models and humans. However, evidence supporting the therapeutic efficacy of VitD3 supplementation in human gastrointestinal infection remains limited. Considering this, we investigated the role of VitD3 in an experimental STM infection employing a colitis mouse model to assess its impact on infection severity and systemic and enteric immune dynamics. **Materials and Methods.** In detail, 6–8-week-old male C57BL/6 mice were fed *ad libitum* for 7 weeks with (Group A, n=15) or without (Group B, n=15) supplementation of VitD3 (75,000 IU/kg). Following 24h of streptomycin treatment, 10 mice from each group were orally infected with 10^4 CFU/0.2 ml of the STM wild-type strain 14028, obtaining Group A1 and Group B1. Uninfected groups (A3 and B3, 5 mice each) remained as controls. Mice were euthanized 3 days post-infection and samples were collected for: complete blood counts (CBC, flow cytometry); bacterial colonization (CFU counts) and IL-1alfa expression in spleens (qPCR); semi-quantitative histological assessment of colitis (proximal

colon sections). Comparison between groups A1 and B1 was made by T-test, with a significance set if $p \leq 0.05$. **Results.** Our results show that A1 mice exhibited significantly improved survival rate and reduced splenic STM colonization and inflammation, compared to B1 mice. Flow cytometry showed a significant reduction in $CD8^+$ T-cells ($CD3^+CD4^-CD56^-$), macrophages ($F4/80^+$), and neutrophils ($CD11b^+$ $Ly6g^+$) in A1 compared to B1 mice, besides a slight increase in B lymphocytes ($B220^+$). Colitis was focal (28.6%) or diffuse (71.4%) and moderate in A1 mice, while all B1 mice showed diffuse severe lymphoplasmacytic infiltration. **Discussion and Conclusions.** These preliminary findings strongly suggest that VitD3 supplementation reduces the severity of STM infection by decreasing bacterial colonization and general inflammation in mice, as evidenced by lower splenic CFU counts, reduced splenic and enteric inflammation, and altered immune cell profiles. The observed reduction in inflammatory immune cells and the slight increase in B lymphocytes suggest that VitD3 modulates the immunity to favour infection control systemically and locally. These results highlight the role of VitD3 as an effective immunomodulatory molecule in combating STM infection and encourage its use as a complementary therapeutic agent for treating human gastrointestinal infections.

147 - Bacterial Extracellular Vesicles Modulate Integrin Expression in Respiratory Epithelial Cells: A Potential Mechanism for Barrier Support and Mucosal Homeostasis.

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Bacterial Extracellular Vesicles Modulate Integrin Expression in Respiratory Epithelial Cells: A Potential Mechanism for Barrier Support and Mucosal Homeostasis.

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Introduction: Bacterial extracellular vesicles (BEVs) are nanosized, bioactive particles released by both Gram-positive and Gram-negative bacteria, known to influence host-pathogen interactions and innate immunity. Beyond their immunomodulatory effects on leukocytes, emerging evidence suggests that BEVs may also directly impact epithelial biology. Here, we propose a proof-of-concept study investigating whether BEVs can modulate the expression of integrins in human respiratory epithelial cells, with potential implications for mucosal barrier integrity and tissue homeostasis.

Materials and Methods: BEVs were isolated from a *Lactobacillus fermentum* 18A-TV strain grown on its stationary phase and were accordingly quantified. To evaluate BEVs effects on integrin modulation, we grew respiratory cells at their confluence by *in vitro* mimicking an epithelial barrier. We stimulated A549 epithelial cells with purified BEVs in cell-culture growth medium supplemented with fetal bovine serum (FBS) and without antibiotics for different time

points (1h, 3h and 24h). Then we gently collected BEVs-treated cells to obtain RNA through commercially available extraction kits. Next, we analyzed the expression levels of selected integrin subunits (e.g., ITGA5, ITGB1, ITGB3, ITGAV) by quantitative PCR (qPCR) through the intercalating agent SYBR Green and using *beta-actin* as housekeeping control gene. We particularly focused on primers design in order to deeply investigate BEVs effects on the transcriptional modulation of conserved domains in integrin subunits. Additionally, we used alternative methods, such as immunofluorescence and Western Blot analysis, to evaluate potential differences in integrin expression on the surface of A549 cells and other ones.

Results: These integrins are critical in maintaining epithelial architecture, mediating cell-matrix adhesion, and regulating repair and immune signaling processes. Preliminary observations indicate that BEVs exposure can upregulate or downregulate specific integrin genes in a dose- and time-dependent manner, suggesting a role in the epithelial response to microbial stimuli.

Discussion and Conclusions: Given the central function of integrins in barrier maintenance—particularly in the respiratory and intestinal mucosa—these findings open new avenues to explore BEVs as modulators of epithelial integrity. Such mechanisms may contribute to either protection or dysregulation, depending on the source and composition of BEVs, with potential relevance in contexts such as infection, inflammation, or microbiota-host interactions. Our study aims to further characterize this regulatory axis, integrating transcriptional data with functional assays (adhesion, permeability) to define how bacterial vesicles influence epithelial resilience.

160 - CD38 as a regulator of airway epithelial cell-driven pro-inflammatory and anti-viral response to SARS-CoV-2, influenza and respiratory syncytial virus

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CD38 as a regulator of airway epithelial cell-driven pro-inflammatory and anti-viral response to SARS-CoV-2, influenza and respiratory syncytial virus

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Introduction: The pathogenesis of respiratory viral infections involves an interplay between the detection of pathogen-associated molecular patterns by cellular receptors and the subsequent activation of host immune responses that must be tightly regulated in terms of both magnitude and temporal dynamics. A common feature among major respiratory viruses, such as respiratory syncytial virus (RSV), influenza A virus (IAV) and SARS-CoV-2, is their ability to trigger local immune responses that, when unbalanced, may result in dysregulated activation of inflammatory and antiviral pathways, ultimately contributing to disease severity. CD38 is an ectoenzyme that catalyses the synthesis of cyclic ADPR (cADPR) and is critically involved in various immune processes. In this study, we aim to investigate, *in vitro,* the role of CD38 in the exacerbation of the pro-inflammatory and antiviral response in human airway epithelial cells infected with RSV, IAV and SARS-CoV-2.
Materials and Methods: Human airway epithelial A549 cells were infected with RSV or IAV, and engineered A549 stably expressing the ACE2 receptor were infected with SARS-CoV-2. Cells were treated or not with 100 micromolar of 8-Br-cADP ribose (8-Br-cADPR), a synthetic analogue of cyclic ADP-ribose. Whole-cell lysates were extracted 30 minutes post infection (p.i.) and total RNA at 24 and 48 hours p.i.. Protein expressions were quantified by western blotting and gene expression by real-time-PCR array.
Results: Infection with RSV, IAV, and SARS-CoV-2 triggers a shared early event, the phosphorylation of STAT3, which can be reduced by pretreatment with the CD38 inhibitor 8-Br-cADPR. As the infection progresses, RSV and IAV induce a significant upregulation of CD38 at both the protein and mRNA levels, whereas SARS-CoV-2 shows a minimal effect. Cytokine profiling reveals that SARS-CoV-2 induces a delayed inflammatory and antiviral response

compared to RSV and IAV. Inhibition of CD38-generated cADPR by 8-Br-cADPR, markedly reduces RSV-induced mediators are, and slightly downregulates of genes upregulated by SARS-CoV-2 is also observed. In contrast, the effect of 8-Br-cADPR on IAV infected cells is less pronounced.

Discussion and Conclusions: CD38 is significantly upregulated in response to RSV and IAV infections, whereas its activation is limited during SARS-CoV-2 infection, which is associated to a delayed cytokines response. Inhibition of CD38 reduces STAT3 phosphorylation and downstream cytokine induction, confirming its involvement in the modulation of inflammatory and anti-viral pathways. These findings suggest that CD38 plays a key role in regulating the host response to severe respiratory viral infections by airway epithelial cells, helping to control virus-induced immune-mediated pathology.

161 - Microgravity and HSV-1 infection Cooperatively Regulate HERV Genes and Retroelement-Derived lncRNAs in Human Macrophages

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Microgravity and HSV-1 infection Cooperatively Regulate HERV Genes and Retroelement-Derived lncRNAs in Human Macrophages

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Background:
Environmental stressors such as microgravity can influence immune responses and gene regulation. Human endogenous retroviruses (HERVs), along with long non-coding RNAs (lncRNAs) derived from their long terminal repeats (LTRs), are known to participate in immune modulation and cellular stress adaptation. Yet, how these elements respond to both microgravity and viral infection has not been fully explored.

Objective:
To examine how simulated microgravity and HSV-1 infection—using both wild-type and VP22-deficient strains—affect the expression of endogenous retroviral genes, retroelement-associated lncRNAs, and inflammatory markers in human macrophages.

Methods:
THP-1 monocytes were differentiated into M0 macrophages and cultured under either normal gravity (1g) or simulated microgravity (μg). Cells were then infected with HSV-1 wild-type or VP22-deficient strains. Gene expression of selected lncRNAs, HERV env/gag elements, and cytokines (IL-1β, IL-6) was analyzed by quantitative PCR. Viral replication and expression of immediate-early HSV genes (ICP0, ICP4, ICP27) were also assessed.

Results:
V22-deficient HSV-1 showed impaired viral gene expression and reduced replication, particularly under microgravity. IL-1β and *HERVK-env/gag* expression increased significantly in μg conditions. Several lncRNAs with retroviral origin—including *UCA1, TROJAN, HCP5,* and *AFAP1-AS1*—were upregulated in response to microgravity and/or the mutant virus, with *UCA1* showed the most notable increase across conditions

Conclusion:
In summary, our findings indicate that both viral factors and microgravity may influence the transcriptional landscape of macrophages, particularly by modulating HERV-related elements and their associated lncRNAs. These observations provide preliminary insight into how combined environmental and infectious stressors could shape innate immune responses under space-like conditions.

163 - Investigating the role of CD38 and Nrf2-antioxidant pathway in the inflammatory response to respiratory virus infection: insights from Air-Liquid Interface models.

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Investigating the role of CD38 and Nrf2-antioxidant pathway in the inflammatory response to respiratory virus infection: insights from Air-Liquid Interface models.

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Introduction: The host immune response to respiratory viral infections is characterized by an activation of pro-inflammatory and anti-viral pathways that, if exacerbated, can lead to severe complications. These infections are also associated with a marked increase in oxidative stress and a down modulation of Nrf2-mediated antioxidant response has frequently been observed. CD38, an ectoenzyme upregulated by viral infections, is involved in immune modulation and regulation of intracellular calcium levels. Our hypothesis is that oxidative stress, observed during respiratory viral infections, may be driven, in part, by CD38-mediated NAD^+ depletion and cADPR production, resulting in impairment of Nrf2 antioxidant defense and exacerbation of inflammation. This study aims to explore how CD38 and Nrf2 modulation influence oxidative

stress and inflammation after respiratory viral infections, using air liquid interface (ALI) cell models to mimic human airway physiology.

Material and methods: An in vitro study is being conducted using primary cells of human nasal epithelial (HNEpC), normal human bronchial epithelial (NHBE) or pulmonary alveolar epithelial (PAEpiC) cells, cultured under ALI conditions. The apical compartment of the ALI is infected with human or avian influenza virus strains, respiratory syncytial virus (RSV-A) and seasonal or pandemic coronaviruses. The viral titre is measured by TCID50 assay. Whole-cell lysates, supernatants and total RNA are collected at 24, 48 and 72 hours post-infection (p.i.). Protein expression of CD38, Nrf2 and antioxidant proteins are analysed by western blotting and the relative genes by real-time-PCR assay.

Results: Preliminary data show that influenza type A virus replicates efficiently in differentiated NHBE cells, with the peak viral replication detected at 24 h p.i. In cultures of ALI-infected NHBE cells at 24, 48 and 72 hours p.i., marked up-regulation of CD38 at both the protein and mRNA levels is observed. This upregulation coincides with a decrease in Nrf2 and antioxidant factors at the same time points. Furthermore, the cytokine profile displays an up-regulation of both pro-inflammatory genes such as IL-6 and CCL5, and anti-viral genes such as IFN-beta and ISG15.

Discussion and Conclusions: Early findings support a role for CD38 and Nrf2 in the signature of oxidative stress in the airway epithelium during respiratory viral infection. The planned activities will provide crucial information on the molecular mechanism of CD38 and Nrf2 pathways in response to inflammation and oxidative stress induced by respiratory viral infections. The results obtained could be instrumental to identify potential pathogenetic biomarkers and to explore future potential therapeutic targets.

171 - In Vitro Analysis of HERV-W and Mycobacterium avium subsp. paratuberculosis-derived Peptide Interactions with Human Pancreatic Islets: Implications for Type 1 Diabetes Pathogenesis.

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In Vitro Analysis of HERV-W and *Mycobacterium avium* subsp. *paratuberculosis*-derived Peptide Interactions with Human Pancreatic Islets: Implications for Type 1 Diabetes Pathogenesis

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Introduction: Type 1 diabetes (T1D) results from autoimmune β-cell destruction driven by both genetic predisposition and environmental factors, still the molecular events by which environmental agents trigger islet autoimmunity remain poor understood. Recent studies implicate both Human Endogenous Retrovirus-W envelope protein (HERV-W env) and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) as potential non-classical environmental triggers, but their direct molecular effects on islet biology remain to be characterized. **Materials and Methods:** We performed quantitative secretome proteomics on human pancreatic islets exposed in vitro to HERV-W ENV or MAP peptides. Untreated islets and cytokine-stimulated controls were included. Secreted proteins were quantified by label-free data-independent acquisition (DIA) LC–MS/MS; peptide intensities were $\log_2$-transformed, median-normalized, and imputed using a left-shifted (MinProb) distribution; differential abundance was determined by permutation testing with thresholds of $|\log_2$ fold-change$| > 1$ and FDR ≤ 0.05." **Results:** HERV-W ENV exposure resulted in significant downregulation of extracellular matrix and metabolic proteins, notably XYLT1, APOA4, and SCG3, suggesting impaired structural integrity and metabolism. In contrast, immune-related proteins such as

IGHV4-4 and PSME1 were upregulated, indicating enhanced antigen processing. MAP peptide exposure induced robust upregulation of antigen presentation and matrix remodelling proteins, most prominently CD1C, ADAMTS9, KRT77, and FHAD1, while reducing ECM proteins including VCAN and LAMA4. Cytokine treatment produced a distinct yet overlapping profile, confirming that HERV-W ENV and MAP have unique molecular effects. **Discussion:** The data reveal a quantitative and functional polarization of islet secretome responses: HERV-W ENV and MAP differentially rewire the islet secretome; HERV-W ENV operates as an immune sensitizer, enhancing antigen presentation amidst moderate ECM attenuation whereas MAP driving matrix proteolysis and heightened antigen presentation. The upregulation of CD1C and ADAMTS9 by MAP highlights a mechanism for increased immunogenicity, while HERV-W ENV may promote chronic islet vulnerability. Limited overlap among key effectors (e.g., PSME1, IGHV4-4) suggests convergent sensitization pathways, yet the distinct magnitude and functional clustering of responses underscore non-redundant environmental risk programs. **Conclusion:** This study provides insight into how HERV-W ENV and MAP peptides differentially reprogram the islet secretome. The identification of specific protein signatures offers avenues for biomarker development and highlights the need for targeted prevention strategies in genetically susceptible individuals.

172 - Implementation of immunological markers for the prediction of Epstein-Barr reactivation in transplanted patients

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Introduction. Epstein-Barr virus (EBV) still represents a great concern in solid organ transplant recipients and the major complication is represented by post-transplant lymphoproliferative disorder (PTLD). There is an urgent need of diagnostic algorithms and introduction of potential immunological markers predictive of the risk of EBV reactivation and related diseases. Here, we analysed the incidence of EBV reactivation and immunological parameters in a cohort of kidney transplant recipients (KTRs) enrolled at time of transplant and followed-up for the first year after transplant.

Material and methods. Immunological parameters were evaluated before transplant (time 0) and after 60, 120, 240 and 360 days after transplant. The IFN-γ response was evaluated against three different peptides pools of EBV (EBNA, LMP and lytic) and results were given as IFN-γ spot forming units (SFU)/10^6 PBMC. Additionally, CD4+ cells/µl were determined by flow cytometry. EBV DNA was monitored during the overall period of follow-up. Additionally, occurrence of other viral infections were recorded. Demographic and clinical characteristics of enrolled patients were collected. Healthy subjects were included as controls.

Results. Overall, 25 EBV-seropositive healthy subjects (16 F/ 9M; age 48, range 33-67) and 66 EBV-seropositive KTRs (27 F/ 39 M; median age 51 range 25-73) were included in the study. Median lymphocyte T-cell counts and median EBV-specific T-cell response were significantly higher in healthy controls than respect to patients at time of transplant ($p<0.01$).

During the follow-up period, 38/66 KTRs (57.6%) developed at least one episode of EBV DNAemia (median 1070 IQR 292-2910 copies/mL) at median 44 days after transplant (IQR 20-150 days); additionally, 57/66 KTRs (86.4%) showed at least one episode of CMV DNAemia (median 20750 copies/mL IQR 5085-323700 at median 71 days after transplant IQR 49-93 days). Other infections (e.g HSV1, VZV, BK and JC) were recorded in 29/66 (43.9%) patients.

Looking at immunological response, patients were classified in two groups according with the level of CD4+ cells/µl at 2 months after transplant. In detail, 11/59 patients had CD4+ cells/ µl≤200 and 48/59 had CD4+ cells/ µl>200. Interestingly, 82% of patients of group 1 developed at least one episode of positive EBV DNAemia while only 54% of patients of group 2 had a detectable EBV DNAemia event during the follow-up ($p=0.092$).

Discussion and Conclusions. Immunological markers could be used for prediction of the infectious risk and, specifically, of EBV reactivations**.** Prospective evaluation of EBV-specific T-cell response will be analysed in order to combine several immunological markers and generate a potential immunological algorithm for management of transplanted patients.

173 - Herpesvirus and Adenovirus Infections: Associations with Frailty, Inflammation, and Functional Decline in Older Adults

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Herpesvirus and Adenovirus Infections: Associations with Frailty, Inflammation, and Functional Decline in Older Adults

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Introduction: Geriatric frailty is characterized by multisystem decline leading to increased morbidity and mortality. Viral infections with Herpesviruses and Adenoviruses may contribute to this process. This study investigates the prevalence of HSV-1, CMV, EBV and VZV among Herpesviruses and HAdV-B (Adenovirus), and their association with frailty, mortality, immunodepression, inflammation, and physical function in an elderly cohort.

Materials and Methods Within the framework of the VIROMA project, 456 geriatric patients (aged 60 to 102 years) who were hospitalized at IRCCS INRCA between 2012 and 2017 were included in the study. Viral DNA was extracted from plasma samples using commercial kits and analyzed by real-time PCR with virus-specific primers. Statistical analyses were performed using logistic regression, Cox proportional hazards models, and linear regression models, adjusted for age and sex.

Results: The prevalence of CMV was 5.2% in subjects aged 60–79 years, 5% in those aged 80–89 years, and 7.4% in those aged 90–102 years. HSV-1 prevalence was 10.4% (60–79 years), 7.5% (80–89 years), and 6.2% (90–102 years). EBV was detected in 1.5% of patients aged 60–79 years, 4.1% of those aged 80–89 years, and was absent in those over 90 years. HAdV-B prevalence was 3% (60–79 years), 2.1% (80–89 years), and 6.2% (90–102 years). VZV was the most frequent infection, with prevalences of 23.1%, 18.3%, and 23.5% across the respective age groups.
EBV was significantly associated with lower frailty (OR=0.228; 95% CI: 0.07–0.74; p=0.014) and an increased risk of immunodepression (OR=8.50; 95% CI: 1.65–43.68; p=0.010). CMV infection was associated with impaired instrumental activities of daily living (IADL; Beta=0.130; p=0.006) and HSV-1 infection with impaired basic activities of daily living (ADL; Beta=0.104; p=0.024). VZV infection was associated with a reduced risk of heart failure (OR=0.417; 95% CI: 0.18–0.97; p=0.042). No significant associations were found between viral infections and 1-, 3-, or 10-year mortality. Regarding inflammatory biomarkers, CMV was associated with increased erythrocyte sedimentation rate (ESR) (p=0.001), HSV-1 with elevated IL-6 levels (p<0.001) and α2-macroglobulin (p<0.001), and HAdV-B with elevated α2-macroglobulin (p=0.037).

Discussion and Conclusions: Herpesvirus and Adenovirus infections selectively influence clinical aspects of geriatric frailty without impacting overall mortality. EBV appears protective against frailty but is associated with higher immunodepression risk, while VZV correlates with a lower risk of heart failure. Conversely, CMV and HSV-1 impair physical function and promote systemic inflammation. These findings suggest that viral infections may contribute to functional decline and chronic inflammation in older adults, highlighting the need for further prospective and mechanistic studies.

187 - A Complex Case of Visceral Leishmaniasis with Extrapulmonary Tuberculosis Infection in a 57-Years-Old Patient

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Background: Hepatitis C virus (HCV) is an RNA virus responsible for chronic liver diseases, including cirrhosis and hepatocellular carcinoma. The virus exhibits high genetic variability, leading to the classification of HCV into 8 major strains and numerous subtypes. The genetic heterogeneity of HCV has significant clinical implications, particularly in terms of the virus's response to antiviral therapies. These viral genotypes are differently distributed around the world, with a prevalence in Europe of type 1. Although new direct-acting antiviral therapies (DAAs) have demonstrated improved efficacy across a wider range of genotypes, the presence of specific mutations within these strains can still affect treatment outcomes and contribute to viral resistance. Understanding the global distribution and characteristics of different HCV strains is critical for optimizing diagnostics, treatment regimens, and public health strategies.

Methods: We conducted a monocentric observational study from 2019 to 2024 at the University Hospital R. Dulbecco of Catanzaro. HCV RNA was quantified using real-time PCR, detection limit of 15 UI/ml. Genotyping and subtyping were carried out by the Versant HCV genotype v2.0 assay (LiPA).

Results: During the study period, 67.091 consecutive new subjects were screened for HCV infection. Among them, 3.4% of patients tested positive for HCV Ab. This prevalence remained stable during the 5-year period (2019-2024) with a heterogeneous distribution between males and females (59% and 40% respectively). Genotyping was performed on 279 sera (12%) from as many viremic patients. The most prevalent genotype/subtype was HCV1b (33%) and HCV2a/2c (25%). The pattern of HCV genotype/subtype prevalence shown differences on the gender and age of population. HCV2a/2c subtype is more prevalent in females at 36% compared with 15% in males, in contrast HCV3 subtype is more frequent in males (20%) than in females (9%). HCV1/1a/1b, HCV2, and HCV4 genotype distribution did not display significant differences between genders. In addition, most of the women who had detectable HCV RNA were born before 1949, while for men the average age is lower, peaking in the 1960-

1969 decade, demonstrating a substantial difference of about 20 years. HCV1b genotype/subtype is most represented regardless of patient age, maintaining the same trend over the 5-year period with a slight decrease in younger subjects. For patients born before 1949, the HCV2a/2c genotype results in the highest expression. In contrast, in later generations there is a 23% increase in people infected with the HCV3 genotype.

Discussion: The observed decrease in HCV Ab seroprevalence could be considered the result of combined actions implemented to eradicate HCV infection (the screening campaigns or the increased availability of new DAA drugs). The alternating prevalence of some HCV genotypes could indicate that this evolution may be independent of the advent of pan-genotypic treatments, but more to epidemiological transmission routes, which should be controlled. In conclusion, epidemiological analyses are important to understand the evolution of HCV epidemics under the influence of several factors, including age and sex. The decrease in HCV Ab prevalence demonstrates the containment of the virus. It is important to monitor the relative prevalence of HCV genotypes so as to understand and monitor possible emerging transmission routes.

231 - The role of sex in prenatal immunity response: divergent modulation of foetal responses to bacterial infection between males and females.

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The role of sex in prenatal immunity response: divergent modulation of foetal responses to bacterial infection between males and females.

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Introduction: Sex-based differences influence the inflammatory response in adults, with females generally exhibiting more active immune reactions and increased secretion of pro-inflammatory cytokines compared to males. Although similar differences in infection susceptibility have been observed in early life, limited research has focused on the sex-specific foetal immune response to intra-amniotic infections. Our previous results have shown that female foetuses exhibited stronger cytokine response during mid-pregnancy than males upon *Mycoplasma hominis* infection, suggesting that sex-specific differences in inflammatory responses could influence maternal health and foetal development.

Materials and Methods: We developed an experimental model to assess innate immune responses in male and female amniotic fluid cells (AFCs) following infection with clinical strains of *Escherichia coli* and *Streptococcus agalactiae,* key microorganisms involved in intra-amniotic inflammation/infections. We analysed both gene and protein expression levels of key pro-inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-alpha which are known to play roles in preterm labour. Additionally, we examined expression of TLR-2 and TLR-4, and selected miRNA profiles in infected male and female AFCs.

Results: Infected male and female AFCs showed more abundant cytokine mRNAs according to bacterial infections. Moreover, we found more actively transcribed *TLRs* in female AFCs infected with *S. agalactiae*. Interestingly, miRNAs expression revealed differences between male and female AFCs upon bacterial infection, finely regulating proteins production.

Discussion and Conclusions: Our findings suggest a divergent modulation of foetal immune response between males and females linked with bacterial injury, suggesting that both sex-specific differences and the bacterial species involved in infection could influence maternal health and foetal development. These variations may contribute to differential foetal programming, potentially affecting long-term health outcomes.

234 - Mucosomes: A new ally against bacterial infections in macrophages

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Mucosomes: A new ally against bacterial infections in macrophages

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Introduction. Respiratory tract infections, such as pneumonia and bronchiolitis, constitute a primary cause of mortality on a global scale. The etiological agents responsible for these infections are bacteria and viruses. Following their entry into the human host's airways, these pathogens are detected by the innate immune system's first defense: alveolar macrophages. These macrophages phagocytize the pathogens, subsequently recruiting and activating other immune cells. Given the frequent ineffectiveness of treatments that rely on antibacterial and antiviral medications, there is an urgent need for alternative or complementary strategies, as well as an efficient nanocarrier capable of delivering therapeutic agents to the site of infection. Mucosomes, a type of glycosylated mucin-based nanoparticles, have been identified as a promising nanocarrier for drug delivery in respiratory airways due to their capacity to bind

glycan-binding proteins expressed by pathogens and immune cells, thereby facilitating targeted delivery of therapeutic agents to the infection site. **Materials and Methods.** A murine macrophage cell line was used first to evaluate the biocompatibility of nanoparticles (both empty and drug-loaded) in a dose/time dependent manner; and second to establish a 2D cell infection model using a Gram-negative bacterial strain. The infection was conducted in a dose/time-dependent manner. The viability of macrophages was determined, and their phagocytic activity was evaluated by lysing cells and quantifying the internalized bacteria. After establishing the appropriate conditions, the empty and drug-loaded mucosomes were introduced into the 2D cell infection model. Confocal microscopy images of infected macrophages were acquired. **Results.** The preliminary results demonstrated that both empty and drug-loaded mucosomes were biocompatible with macrophages. Furthermore, the drug-loaded exhibited antibacterial properties. In a more complex cell infection model, the results demonstrated that the phagocytic activity of macrophages is enhanced by drug-loaded mucosomes. **Discussion.** Given the preliminary results of the present work, mucosomes represent a valuable and efficient nanocarrier to deliver the therapeutic agents into the respiratory airways at the site of infection. The glycan-binding proteins present on mucosomes facilitate the binding of pathogens and immune cells, thereby enhancing immune cell phagocytic activity. **Conclusions.** Mucosomes are efficient drug-delivery nanocarriers since they pass through the mucus layer and reach the targeted site. They represent a powerful nanosystem for nanomedicine, particularly in the context of immune cell phagocytosis. Further biological studies will be conducted to assess the potential of drug-loaded mucosomes.

243 - Learning from COVID-19: tailoring vaccine strategies for immunocompromised populations

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Learning from COVID-19: tailoring vaccine strategies for immunocompromised populations

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Introduction. Patients with primary and secondary immunodeficiencies, being more susceptible to infections, are a priority for vaccination. To profile the immune response elicited by SARS-CoV-2 vaccination in different cohorts of immunocompromised patients, we have conducted a longitudinal prospective single-center study.

Materials and Methods. The PATO_VAC study was conducted at Siena University Hospital to characterize the spike-specific B cell and antibody responses to SARS-CoV-2 mRNA vaccination in a total of 590 patients, including hematopoietic stem cell and solid organ transplant recipients (SOT), patients with haematological malignancies, on haemodialysis and people living with HIV (PLWH). Subjects were followed up for two years after vaccination.

Results. A different immune responsiveness was observed after the first two doses, with hematological, hemodialysis and solid organ transplanted subjects less responsive than stem-cell transplanted patients and PLHW. Spike-specific B cell response was slower and lower in all cohorts except in PLWH, but the first booster dose strongly increased both the B and the antibody responses, that persisted up to 2 years after the first vaccine administration. Patients persisting with a low response, especially among SOT, were detected. **Discussion and Conclusions**. Our study highlights the heterogenicity of immune response in immunocompromised patients, the pivotal role of the third dose, and the persistence of low-responders subjects despite multiple vaccine administrations. These aspects should be carefully considered for designing vaccination schedules specifically tailored for patients with different immunocompromising pathologies.

260 - West Nile Virus Infection and Persistence in Murine Macrophages

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West Nile Virus Infection and Persistence in Murine Macrophages

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Introduction and Aims:

Arboviruses such as West Nile virus (WNV) pose a significant and growing threat to global public health due to their capacity to establish persistent infections in both avian reservoirs and mammalian hosts. WNV, predominantly transmitted by *Culex* mosquitoes, is associated with severe clinical outcomes, including neuroinvasive disease and renal impairment. Despite extensive research, the molecular and cellular mechanisms underlying viral persistence remain largely elusive. Macrophages, as key components of the innate immune system and potential reservoirs for viral replication, play a central role in shaping the course of WNV infection. This study aims to investigate the contribution of murine macrophages to WNV infection dynamics and viral persistence, employing both *in vivo* and *ex vivo* experimental models.

Methods:

To explore the role of macrophages in WNV pathogenesis, we utilized C57BL/6 mice in a two-pronged approach combining systemic and localized macrophage depletion with subsequent viral infection. Mice were treated with clodronate liposomes (16 mL/kg) either intraperitoneally or via footpad injection six days prior to WNV challenge, in order to selectively deplete total or tissue-resident macrophages, respectively. Post-infection, viral loads were quantified in key target organs—including kidney, spleen, and brain—using qPCR. Parallelly, *ex vivo* experiments were performed using murine monocyte-derived macrophages (MDMs), which were infected with WNV and monitored over time (4, 8, and 12 days post-infection) to assess viral replication, cytopathic effects, and immunofluorescence-based detection of viral antigens.

Results:

Preliminary data demonstrate that intraperitoneal administration of clodronate liposomes effectively reduced both total and resident macrophage populations in the peritoneal cavity when compared to PBS-treated controls. In contrast, conclusive depletion of resident macrophages in the footpad skin could not be confirmed, potentially affecting viral kinetics at the local site of infection. Since the footpad was also the site of WNV inoculation, macrophage presence in this tissue may be particularly relevant for understanding viral entry and

persistence mechanisms. *In vivo* depletion of macrophages led to marked changes in tissue-specific viral distribution, suggesting that macrophage abundance and localization influence WNV dissemination and organ tropism.

Conclusion:

Our findings underscore the pivotal role of macrophages in modulating WNV infection and persistence. Both *in vivo* and *ex vivo* results suggest that macrophage depletion alters viral replication dynamics, highlighting their potential as targets for therapeutic intervention. Ongoing studies are focused on the role of N-acylethanolamine acid amide hydrolase (NAAA)—an enzyme involved in macrophage sphingolipid metabolism—in influencing WNV replication and immune evasion. Targeting NAAA activity may offer a novel antiviral strategy not only against WNV but also against a broader range of persistent arboviral infections.

261 - SARS-CoV-2 Variants: Pathogenicity and Host Immune Response in K18-hACE2 mice

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TOPIC: Risposta immunitaria dell'ospite alle infezioni

TITLE: SARS-CoV-2 Variants: Pathogenicity and Host Immune Response in K18-hACE2 mice

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Introduction: The clinical manifestation of COVID-19 has evolved significantly throughout the pandemic, shifting from severe, often fatal pneumonia to a more manageable, multi-organ infection. This change reflects advancements in medical knowledge, improved treatments, and widespread vaccination efforts. However, the contribution of viral evolution, including reduced virulence to evade immunity and enhanced transmissibility, remains under investigation. This study utilizes the K18-hACE2 mouse model that replicates key features of human COVID-19, demonstrating a dose-dependent progression from asymptomatic or mild disease at lower viral doses to severe, lethal outcomes at higher doses. The study compares eight representative SARS-CoV-2 variants to identify and characterize biomarkers of pathogenicity and host responses. **Materials and methods:** Mice were infected intranasally with increasing doses of SARS-CoV-2, while sham-infected animals received PBS. Daily monitoring of weight loss and symptoms, along with Kaplan-Meier survival analysis, revealed significant weight loss

correlating with poor survival. Following determination of the optimal viral dose, mice were sacrificed at various time points to evaluate disease progression. **Results:** Viral load was highest in the lungs and brain. Lung pathology, assessed via immunohistochemistry, revealed intra-alveolar hemorrhages, edema, pneumocyte hyperplasia, congestion, inflammation. and multinucleated giant cells. Lipid inflammatory markers were analyzed using MEPS-UHPLC-MS/MS on plasma samples collected at different time points. Of 58 markers analyzed, 50 were detectable in ≥50% of samples and subjected to statistical analysis, revealing significant shifts. Brain tissues from infected and control mice were analyzed for neurodegenerative markers. IFN-I/III gene expression in lung and spleen cells was assessed at four post-infection time points to investigate innate immune responses. **Discussion and Conclusions:** This study provides a comprehensive analysis of SARS-CoV-2 variant pathogenicity and host responses using the K18-hACE2 mouse model. By elucidating the mechanisms of disease progression and immune response, these findings deepen our understanding of COVID-19 and its evolving clinical manifestations. Ongoing investigations aim to identify differences in innate immune responses among SARS-CoV-2 variants.

266 - Investigating the Unfolded Protein Response in HSV-1 infection: A dual role in cellular defence and viral replication

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Investigating the Unfolded Protein Response in HSV-1 infection: A dual role in cellular defence and viral replication

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Introduction: Viral production in host cells leads to Endoplasmic Reticulum stress, triggering the Unfolded Protein Response (UPR). This mechanism work to manage protein folding, reduce translation, and may induce apoptosis. While increased folding capacity can aid viral replication, the translation inhibition and apoptosis can limit viral spread. Many viruses exploit the UPR for their survival, but it also plays a crucial role in enhancing the host's antiviral defences, particularly through interaction with pathogen recognition systems. However, the overall impact of these cellular responses on viral infection outcomes remains poorly understood. Since Herpes Simplex Virus type-1 (HSV-1) infection has an important impact on the nervous system and the UPR is dysregulated in many diseases such as neurodegeneration, this study aims to explore the role of the UPR in herpetic infections using neuron-like cells.

Materials & methods: Human neuroblastoma cell line SH-SY5Y was used for this study. After differentiation with Retinoic Acid (10mM), the cells were infected with 1 MOI (multiplicity of infection). To investigate the role of UPR, cells were treated with Thapsigargin (Tg) 100 nM, an ER stress inducer. The presence of viral genome in the supernatants of infected cells were analysed by plaque assay and RT-PCR. The quantification of the mRNA genes of interest was carried out by RT-PCR.

Results: HSV-1 replication appears to increase following Tg treatment 6 hours before HSV-1 infection. Activation of the UPR could increase the number of cell surface receptors and chaperones that HSV-1 uses as tools to aid its binding to the host cell and entry (Fig1). Conversely, when the UPR was activated 1 hour after infection, viral yields and viral mRNA were significantly reduced. Surprisingly, the reduction in viral replication did not depend on the INF-β response (Fig2).

Conclusions: Taken together, these data suggest that the UPR has a dual role in viral infection. Although the UPR may facilitate the production of new viral particles, it also activates the immune response to counteract viral replication. The results could provide clues to the mechanism of HSV-1-induced neuropathogenesis, and the UPR could be considered a possible target to modulate or inhibit viral infection. Future experiments will explain how the UPR modulates the HSV-1 infection process.

267 - Antiviral properties of human milk: focus on respiratory viruses

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Antiviral properties of human milk: focus on respiratory viruses

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Introduction and aims: Human milk confers protection against respiratory infections, but the mechanisms by which viral neutralization is implemented are not fully known. The aim of the study was to determine the neutralizing ability of colostrum and mature human milk against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and human Adenovirus C.1 (HAdV), defining the role of the specific IgG, and the nonspecific molecules, such as the fatty acids and the human milk sialylated oligosaccharides (HMO).

Materials and Methods: Milk was collected from 74 women, from 4 to 360 days postpartum. SARS-CoV-2 IgG were quantified by means of ELISA test; anti SARS-CoV-2, and anti HAdV neutralizing activity (NT_{50}) was evaluated using the $TCID_{50}$ method, upon *in vitro* infection of permissive cell lines (Vero E6 and A549, respectively). Fatty Acids composition was defined by means of gas chromatography separation and analyses. HMO antiviral activity was tested *in vitro,* in Calu3 cell line. Correlation analysis between antibody response or fatty acids composition and NT_{50} was carried out.

Results: Anti-SARS-CoV-2 IgG were present in 72% of the collected milk samples. SARS-CoV-2 NT_{50} values were higher ($p<0.05$) in colostrum than in mature milk samples, but correlation

between NT_{50} titers and anti-SARS-CoV-2 IgG presence was found only in the mature milk samples from vaccinated mother. The concentration of lignoceric acid and nervonic acid correlated (0.3 and 0.36 respectively) with SARS-CoV-2 NT_{50} values. *In vitro* SARS-CoV-2 replication decreased by 20-50% upon HMO treatment in Calu3 cell line.

Discussion and Conclusions: Human milk showed different behavior against SARS-CoV-2 and HadV, probably due the characteristics of the enveloped and naked virion, respectively. Anti-SARS-CoV-2 IgG were present in most of the collected samples but correlated with the neutralizing activity only in mother subjected to recent vaccination: aspecific neutralizing activity was probably due to other components, such as lignoceric, nervonic acid and/or HMO. Thus, synergistic effect of all the components of the human milk matrix is essential in order to ensure a protection against SARS-CoV-2.

Future perspectives: Test the neutralizing activity of human milk against others enveloped and naked respiratory viruses, such as Rhinovirus, seasonal Influenza virus A and B as well as others SARS-CoV-2 variants, defining the role of specific antibodies and nonspecific molecules.

274 - Herpes simplex virus-1 infection promotes complement activation and microglial synapse pruning in brain cells

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Herpes simplex virus-1 infection promotes complement activation and microglial synapse pruning in brain cells

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Introduction
Accumulating evidence suggests recurrent herpes simplex virus-1 (HSV-1) infection reaching the brain as a risk factor for Alzheimer's disease (AD). Dysregulation of the complement cascade has been linked to AD pathogenesis and synaptic pruning. Thus, we investigated if HSV-1 triggers complement upregulation and promotes synapse loss via microglial phagocytosis.
Materials and Methods
Murine primary neuronal cultures, cocultured or not with BV2 microglia, and organtypic hippocampal brain slices were used as *in vitro* and *ex vivo* infection models. To inhibit complement cascade activation, cultures were treated with a neutralizing anti-C3 antibody (C3Ab) within the infection. Cells and brain tissues were analyzed 24h and 48h post (p.i.) HSV-1 or mock infection with RT-PCR, Western Blotting, confocal immunofluorescence (IF), and virological assays. For the engulfment assay, synaptosomes isolated 24h p.i. were tested for

complement protein presence and layered onto BV2 cells to analyze their engulfment by IF. IF was also exploited to assess microglial synaptic pruning in both infection models, quantifying the colocalization between $CD68^+$ (i.e., microglial lysosomes) and $PSD95^+$ or $EGFP^+$ (i.e., synaptic materials) signals. Organotypic hippocampal slices were biolistically transfected with EGFP plasmid, treated with C3Ab or vehicle, then mock- or HSV-1-infected. Two-photon live imaging and/or IF were performed 48h p.i., quantifying dendritic spine density on secondary apical dendrites of CA1 pyramidal neurons, and microglial synaptic pruning.

Results

We found that HSV-1 increased the expression of the complement components C1q, C4 and C3 at protein and mRNA levels, and promoted the extracellular release of C3 in primary brain cultures. Interestingly, C1q and C4 were found localized at synaptic sites 24h p.i., suggesting that these proteins could represent a tag of those synapses that should be eliminated. Consistently, HSV-1 caused PSD-95 downregulation that was partially rescued in C3Ab-treated cells. Moreover, results from the engulfment assays and IF analyses revealed enhanced microglial phagocytosis of synaptic materials post-HSV-1 infection, that was prevented when the complement cascade was inhibited. Analyses on organotypic hippocampal slices confirmed that the virus triggers synaptic damage through microglial synaptic pruning via a complement-dependent mechanism. Importantly, in infected CA1 neurons we detected a significant decrease in spine density, which was rescued when the infection was performed in the presence of C3Ab. Overall, our data indicate that HSV-1 infection triggers aberrant complement activation and microglial engulfment of complement-tagged synapses in primary brain cultures, further supporting its role in neurodegeneration.

279 - Nuclear Trafficking of Anelloviridae capsid protein ORF1 Reflects Modular Evolution of Subcellular Targeting Signals

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Nuclear Trafficking of *Anelloviridae* capsid protein ORF1 Reflects Modular Evolution of Subcellular Targeting Signals

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1. Introduction. *Anelloviridae* members are ubiquitous viruses establishing long lasting persistent infections in serval mammalian hosts. They are endowed with a small, negative-sense, single-stranded DNA genome that is replicated by host cell DNA polymerases and are therefore postulated to interact with the host cell nuclear transport machinery. However, the lack of reliable cell culture models limits our knowledge regarding Anelloviridae-host interactions, and capsid nuclear import is a largely uncharacterized process.

2. Methods. We combined phylogenetic (Clustal Omega) and bioinformatics (cNLSmapper) analyses with structural modelling (AlphaFold 3), biochemical assays (Electrophoretic Mobility Shift Assays and Fluorescence Polarization Assays) and quantitative imaging (confocal laser scanning microscopy) to investigate the relationship between host cell nuclear transport receptors (NTRs) and ORF1, the putative capsid protein from torque teno douroucouli virus (TTDoV).

3. Results. We identified the subcellular targeting signals and NTRs responsible for its nucleolar and nuclear localization, and characterized their relative contribution to ORF1 subcellular localization. In the absence of other viral proteins, ORF1 accumulated in the nucleoli. A bioinformatics analysis revealed a putative nuclear localization signal ("NLSn", 27-RRWRRRPRRRRRPYRRRPYRRYGRRRKVRRR-57) within the highly conserved N-terminal arginine-rich motif (ARM), and an additional C-terminal ("NLSc", 632-LPPPEKRARWGF-643), which has been specifically acquired by Anelloviridae capsids endowed with larger projection domains, whose presence has been recently implicate in host-immune evasion. Such NLSs

contribute differently to ORF1 subcellular localization. NLSn is endowed with broad importins (IMPs) binding properties, but plays a minor role in nuclear import, being responsible for nucleolar targeting, most likely due to interaction with nucleolar components. NLSc specifically interacts with IMPalpha and is the main driver of active nuclear transport in an IMPalpha/beta1-dependent fashion.
4. Discussion and Conclusions. Out findings suggest an evolutionary correlation between the acquisition of progressively larger projection domains and of additional NLSs in Anelloviridae capsids, aimed at maximizing nuclear import possibilities as mediated by IMPalpha/beta1.

284 - HERV EXPRESSION CORRELATES WITH LYMPHOCYTE ACTIVATION MARKERS IN BLOOD AND GALT OF VIROLOGICALLY SUPPRESSED HIV-1 INDIVIDUALS

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Title: HERV EXPRESSION CORRELATES WITH LYMPHOCYTE ACTIVATION MARKERS IN BLOOD AND GALT OF VIROLOGICALLY SUPPRESSED HIV-1 INDIVIDUALS

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Background: Despite the efficacy of antiretroviral therapy (ART), many people living with HIV-1 (PLWH) show persistent immune activation and harbour latent viral reservoirs that complicate long-term disease management. Several studies have shown that the viral reservoir of the intestinal mucosa is established early in HIV infection and that the gut-associated rectal lymphoid tissue (GALT) represents the main reservoir of HIV, with high levels of viral replication due to massive CD4+ T-cell depletion. Human endogenous retroviruses (HERVs), relics of ancestral exogenous retroviral infections and comprise 8% of the human genome, have been implicated in various chronic inflammatory and infectious diseases, including HIV-1 infection. Indeed, we have already demonstrated the interconnection of human endogenous retroviruses (HERVs) with persistent immune activation in virologically suppressed PLWH. Our aim was to evaluate the transcriptional activity of HERVs, along with immune activation and HIV reservoirs in blood and GALT, in virologically suppressed PLWH, in order to identify markers for a new approach to clinical monitoring. **Material and methods:** 31 HIV-1+ viral suppressed patients, and 10 Healthy Donors (HD) were enrolled. Blood and GALT HIV-DNA levels and residual

plasma viremia were quantified by droplet digital-PCR system. The expression of the env gene of two elements of the HERV-K113 and HERV-K111, of pHERV-W, Syncitin-1 (SYN-1) and HERV-H have been analysed from blood samples by RT-Real time PCR. The immunophenotyping have been evaluated by flow cytometry. The non-parametric Mann-Whitney test and the Spearman Correlation were used for statistical analysis. **Results:** in virologically suppressed PLWH, HERV-K113, HERV-K111 and pHERV-W were significantly high expressed compared to HD. HERV-K113 and HERV-K111 levels correlated positively with blood HIV-DNA and CD8+ T-cell and CD19+ B-cell activation markers in the peripheral blood district, whereas they correlated inversely with CD4 count and nadir. Furthermore, the activation of HERVs and immune system observed in the peripheral blood was found to correlate directly and significantly with the immune activation measured in GALT. Notably, HERV-K113, HERV-K111 and pHERV-W expression positively correlated with CD38 and HLADR activation markers in CD4 and CD8 lymphocytes in GALT. **Conclusions:** The analysis suggests that HERV expression may play a role in immune dysregulation and viral persistence in people living with HIV (PLWH). Immune activation in both blood and GALT highlights a systemic connection between peripheral and mucosal immunity. Monitoring HERV expression could offer valuable insights into chronic inflammation and HIV latency, potentially driving the development of targeted therapies.

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296 - Effect of Simulated Microgravity on the Inflammatory Immune Response of Macrophages Stimulated by bacteria

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Effect of Simulated Microgravity on the Inflammatory Immune Response of Macrophages Stimulated by *bacteria*

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Introduction: Microgravity conditions, such as those experienced by astronauts during prolonged space missions, are known to impair immune system function, increasing susceptibility to infections and other immune-mediated disorders, particularly upon re-entry into Earth's gravity. Macrophages play a crucial role in the early immune response to microbial invasion. In this study, we employed an in vitro model to investigate the effects of simulated microgravity on the pro-inflammatory response of human macrophages stimulated with various bacterial species, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Materials and Methods: THP-1 monocytes were differentiated into macrophages using phorbol 12-myristate 13-acetate (PMA). The differentiated cells were then incubated for 6 hours with *E. coli*, *S. aureus*, or *P. aeruginosa* under simulated microgravity conditions using a clinorotation-based system. Total RNA was extracted, and the expression levels of selected

pro-inflammatory genes were analyzed and compared with those of control cells maintained under standard gravity. In parallel, the effect of microgravity on bacterial proliferation was assessed.

Results: Our results revealed a significant increase in the growth rates of all tested bacterial species under simulated microgravity compared to standard gravity controls. Additionally, we observed differential modulation of pro-inflammatory gene expression in macrophages exposed to microgravity conditions.

Discussion: These findings suggest that microgravity alters host–pathogen interactions, potentially through changes in nutrient diffusion, gene regulation, and/or bacterial replication dynamics. Moreover, the innate pro-inflammatory response of human macrophages to bacterial stimuli is modulated by microgravity, highlighting the need to monitor immune function in astronauts during space missions. The molecular mechanisms underlying the altered immune response observed in microgravity remain to be fully elucidated.

297 - Phenotypic Comparison of Escherichia coli and Pseudomonas aeruginosa in a Galleria mellonella Burn Wound Model

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Phenotypic Comparison of *Escherichia coli* and *Pseudomonas aeruginosa* in a *Galleria mellonella* Burn Wound Model

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Introduction: Burn wound infections are a significant clinical concern, with *Pseudomonas aeruginosa* being a well-established pathogen in this context. *Escherichia coli*, although less frequently isolated, is increasingly recognized as a relevant Gram-negative pathogen in burn wounds. This study employs the *Galleria mellonella* model to compare the phenotypic impact of *E. coli* and *P. aeruginosa* under standardized burn injury conditions.
Materials and Methods: Burn wounds were induced in *G. mellonella* larvae using a calibrated thermal injury device. Post-injury, larvae were topically infected with clinical isolates of *E. coli* (10 µL, 1.3×10^6 CFU). Mortality was monitored at fixed intervals over 48 hours. Phenotypic parameters included Kaplan-Meier survival, melanization, and motility loss. Results were compared to previously published data obtained using *P. aeruginosa* ATCC 27853 in the same experimental setup. All experiments were performed in triplicate.

Results: Preliminary observations confirm the suitability of the *G. mellonella* model for assessing differential virulence traits among Gram-negative pathogens. Visual and survival-based phenotypic endpoints provide measurable distinctions in host response and disease progression. Comparative patterns between *P. aeruginosa* and *E. coli* are under investigation and will be presented, offering insight into relative pathogenic potential in burn wound settings.
Discussion and Conclusions: The findings suggest that *E. coli*, while capable of establishing infection, displays a distinct virulent phenotype compared to *P. aeruginosa* in the *G. mellonella* burn wound model. This phenotypic distinction highlights differences in pathogenic strategies, possibly linked to virulence factor expression. The model confirms its utility for comparative virulence profiling of burn wound pathogens and reinforces the clinical relevance of emerging Gram-negative bacteria beyond *P. aeruginosa*.

298 - Tracking Torque Teno Virus in Periprosthetic Fluids as a sentinel of Local Immune Dynamics

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Tracking Torque Teno Virus in Periprosthetic Fluids as a sentinel of Local Immune Dynamics

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Introduction

The Torque Teno virus (TTV) is an ubiquitous DNA virus, orphan of pathology, part of the human virome and strictly correlated with the immune system; the plasma TTV viral load is between 10^2 and 10^3 copies/mL in the immunocompetent, whereas it increases in cases of immunosuppression.

This study explores a novel context for TTV detection: periprosthetic fluid collected from patients undergoing mastectomy and prosthetic reconstruction following breast cancer surgery. While most studies focus on TTV as a systemic biomarker, we hypothesized that its detection and quantification at the local level could offer insights into peri-implant immune dynamics, particularly in the early postoperative period, vulnerable to complications such as infection and delayed healing.

Given that postoperative infections are known to adversely affect reconstructive outcomes and oncological follow-up, identifying molecular markers of early inflammatory activity is of critical importance. In this framework, we propose that TTV may serve as a sentinel virus, reflecting the immunological state of the periprosthetic microenvironment.

Materials and methods

Periprosthetic fluid samples (n=48) were collected at different times in the early postoperative period, and TTV DNA was detected and quantified using real-time PCR. Clinical data were correlated with local viral load to explore associations with patient immune status and potential infectious or inflammatory complications.

Results

TTV DNA was detected in 79.2% of periprosthetic fluid samples, with viral loads ranging from 1.00 to 6.75 $\log_{10}$ copies/mL (mean: 4.06). Of interest, non-smokers exhibited significantly higher TTV loads than smokers (median: 4.79 vs 3.58 $\log_{10}$ copies/mL), suggesting that chronic immune activation in smokers may suppress viral replication.

TTV load also showed correlation with local immune cell presence, markedly higher in smokers (2:1 ratio vs 0.75:1 in non-smokers), in the absence of overt infection. Even in the presence of infection, TTV levels are significantly lower in smokers.

Discussion

Data obtained reinforce the role of TTV as a marker of immune activation, even at the local level.

Further studies are warranted to explore the potential of TTV quantification in periprosthetic fluid as a predictor of implant-related complications.

Furthermore, it is imperative to consider smoking status when interpreting TTV levels.

Conclusions

These findings confirm the feasibility to detect TTV in periprosthetic fluid and support its potential role as a sentinel marker of local immune activity. Further studies are needed to validate TTV as a reliable biomarker of periprosthetic immune tone, particularly in clinical settings where conventional inflammatory markers are lacking or inconclusive.

312 - Phenotypic Comparison of Escherichia coli and Pseudomonas aeruginosa in a Galleria mellonella Burn Wound Model

Farwa Mukhtar *(1)* ***- Addis Temie Worku*** *(1)* ***- Antonio Guarnieri*** *(1)* ***- Natasha Brancazio*** *(2)* ***- Marilina Falcone*** *(1)* ***- Maria Di Naro*** *(2)* ***- Marco Allfio Cutuli*** *(1)* ***- Irene Magnifico*** *(1)* ***- Daria Nicolosi*** *(3)* ***- Roberto Di Marco*** *(3)* ***- Giulio Petronio Petronio*** *(1)*

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Phenotypic Comparison of *Escherichia coli* and *Pseudomonas aeruginosa* in a *Galleria mellonella* Burn Wound Model

FARWA MUKHTAR *[1]*, ***ADDIS T. WORKU*** *[1]*, ***ANTONIO GUARNIERI*** *[1]*, ***NATASHA BRANCAZIO*** *[1,2]*, ***MARILINA FALCONE*** *[1]*, ***MARIA DI NARO*** *[1,2]*, ***MARCO A. CUTULI*** *[1]*, ***IRENE MAGNIFICO*** *[1]*, ***DARIA NICOLOSI*** *[3]*, ***ROBERTO DI MARCO*** *[3]* ***AND GIULIO PETRONIO PETRONIO*** *[1]*.

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Introduction: Burn wound infections are a significant clinical concern, with *Pseudomonas aeruginosa* being a well-established pathogen in this context. *Escherichia coli*, although less frequently isolated, is increasingly recognized as a relevant Gram-negative pathogen in burn wounds. This study employs the *Galleria mellonella* model to compare the phenotypic impact of *E. coli* and *P. aeruginosa* under standardized burn injury conditions.
Materials and Methods: Burn wounds were induced in *G. mellonella* larvae using a calibrated thermal injury device. Post-injury, larvae were topically infected with clinical isolates of *E. coli* (10 µL, 1.3×10^6 CFU). Mortality was monitored at fixed intervals over 48 hours. Phenotypic parameters included Kaplan-Meier survival, melanization, and motility loss. Results were compared to previously published data obtained using *P. aeruginosa* ATCC 27853 in the same experimental setup. All experiments were performed in triplicate.

Results: Preliminary observations confirm the suitability of the *G. mellonella* model for assessing differential virulence traits among Gram-negative pathogens. Visual and survival-based phenotypic endpoints provide measurable distinctions in host response and disease progression. Comparative patterns between *P. aeruginosa* and *E. coli* are under investigation and will be presented, offering insight into relative pathogenic potential in burn wound settings.
Discussion and Conclusions: The findings suggest that *E. coli*, while capable of establishing infection, displays a distinct virulent phenotype compared to *P. aeruginosa* in the *G. mellonella* burn wound model. This phenotypic distinction highlights differences in pathogenic strategies, possibly linked to virulence factor expression. The model confirms its utility for comparative virulence profiling of burn wound pathogens and reinforces the clinical relevance of emerging Gram-negative bacteria beyond *P. aeruginosa*.

317 - Altered neutrophil CD45 expression correlates with clinical severity in infants hospitalized for RSV bronchiolitis

Matteo Fracella *(1)* ***- Francesca Pulcinelli*** *(2)* ***- Federica Frasca*** *(1)* ***- Eleonora Coratti*** *(1)* ***- Alessandra D'Auria*** *(1)* ***- Laura Petrarca*** *(2)* ***- Maria G. Conti*** *(2)* ***- Carolina Scagnolari*** *(1)* ***- Raphael P. Viscidi*** *(3)* ***- Raffaella Nenna*** *(2)* ***- Fabio Midulla*** *(2)* ***- Guido Antonelli*** *(1)* ***- Alessandra Pierangeli*** *(1)*

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Altered neutrophil CD45 expression correlates with clinical severity in infants hospitalized for RSV bronchiolitis

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Introduction

Respiratory syncytial virus (RSV) is a leading cause of bronchiolitis in children, with disease severity closely related to dysfunction of the host immune response within the airway microenvironment. Comprehensive characterization of key immune cells, including neutrophils, monocytes, and lymphocytes in the nasal mucosa - the primary site of RSV entry - along with their maturation and activation states, is critical to elucidating their distinct roles in inflammation, tissue injury, and viral clearance. Hence, we investigated cell populations in the nasal mucosa of RSV-positive infants with bronchiolitis during the 2023/2024 season.

Materials and Methods

Nasal wash (NW) samples were collected from 38 infants hospitalized for bronchiolitis during the 2023/2024 epidemic season at the Pediatric Emergency Department of Sapienza University Hospital. All specimens tested positive for RSV using the Xpert® Xpress CoV-2/Flu/RSV rapid molecular assay and/or in-house PCR-based assays. Flow cytometry was performed on fresh cell pellets, which were stained with fluorochrome-conjugated antibodies against CD45, CD14, CD16, CD66b, CD3, and CD326, as well as a viability dye, following standard protocols. Data were acquired using a CytoFLEX flow cytometer and analyzed with CytExpert software v2.4.

Results

Thirty-eight NW samples from RSV-positive infants with bronchiolitis were analyzed, including 23 males (mean age 3.52 ± 2.77 months) and 15 females (mean age 2.93 ± 2.68 months).

Neutrophils (*CD45*$^{+}$*CD16*$^{+}$*CD66b*$^{+}$) represented the predominant immune population, with a median frequency of 85.90% within the gated viable CD45^{+} cell population, followed by non-classical monocytes (*CD45*$^{+}$*CD14*$^{-}$*CD16*$^{+}$), which accounted for 50.84% of the gated viable CD45^{+} cells excluding neutrophils and T lymphocytes. Classical (*CD45*$^{+}$*CD14*$^{+}$*CD16*$^{-}$) and intermediate (*CD45*$^{+}$*CD14*$^{+}$*CD16*$^{+}$) monocytes were less common, at 1.12% and 0.21%, respectively, within the same parent population. Epithelial cells (*CD326*$^{+}$) and T lymphocytes (*CD45*$^{+}$*CD3*$^{+}$) were observed at very low levels, accounting for 2.91% and 2.11% of viable cells, respectively. Neutrophils were predominantly characterized by a *CD16*$^{+}$*CD66b*$^{+}$*SSC*^{high} phenotype, consistent with mature and highly granular cells. However, in infants with greater disease severity, a distinct shift in CD45 expression was observed within the neutrophil population, with a reduction in *CD45*^{high} neutrophils and a concomitant expansion of *CD45*^{low} subsets compared to infants with lower severity scores ($p<0.05$).

Discussion and Conclusions

The alteration observed in infants with greater disease severity may reflect a dysregulated neutrophil response associated with impaired maturation, aberrant activation, and increased inflammatory potential, thereby contributing to enhanced tissue damage and exacerbation of RSV-induced bronchiolitis.

323 - Phenotypic Comparison of Escherichia coli and Pseudomonas aeruginosa in a Galleria mellonella Burn Wound Model

Farwa Mukhtar *(1)* ***- Addis Temie Worku*** *(1)* ***- Antonio Guarnieri*** *(1)* ***- Natasha Brancazio*** *(2)* ***- Marilina Falcone*** *(1)* ***- Maria Di Naro*** *(2)* ***- Marco Allfio Cutuli*** *(1)* ***- Irene Magnifico*** *(1)* ***- Daria Nicolosi*** *(3)* ***- Roberto Di Marco*** *(3)* ***- Giulio Petronio Petronio*** *(1)*

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Phenotypic Comparison of *Escherichia coli* and *Pseudomonas aeruginosa* in a *Galleria mellonella* Burn Wound Model

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Materials and Methods: Burn wounds were induced in *G. mellonella* larvae using a calibrated thermal injury device. Post-injury, larvae were topically infected with clinical isolates of *E. coli* (10 µL, 1.3×10^6 CFU). Mortality was monitored at fixed intervals over 48 hours. Phenotypic parameters included Kaplan-Meier survival, melanization, and motility loss. Results were compared to previously published data obtained using *P. aeruginosa* ATCC 27853 in the same experimental setup. All experiments were performed in triplicate.

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Discussion and Conclusions: The findings suggest that *E. coli*, while capable of establishing infection, displays a distinct virulent phenotype compared to *P. aeruginosa* in the *G. mellonella* burn wound model. This phenotypic distinction highlights differences in pathogenic strategies, possibly linked to virulence factor expression. The model confirms its utility for comparative virulence profiling of burn wound pathogens and reinforces the clinical relevance of emerging Gram-negative bacteria beyond *P. aeruginosa*.

340 - Varicella Zoster Virus Vaccination in patients with haematological diseases and primary immunodeficiencies. Assessment of specific T and B-cell responses

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Introduction:

The reactivation of varicella-zoster virus (VZV) causes herpes zoster (HZ) and is more frequent in subjects with primitive or acquired impairment of the immune system. An adjuvanted recombinant subunit vaccine (HZ/su) has been approved for the prevention of HZ in adults with immune impairments, although limited data on HZ/su immunogenicity in this specific group are available. We aimed to investigate VZV-specific immunity in haematological patients (HE) and individuals with primitive immunodeficiency (PrID) after HZ/su vaccination.

Material and methods:
HE and ID patients were administered two doses of HZ/su 2 months apart (T0 and T1 respectively), with a follow-up visit 1 month after the second dose (T2). T-cell responses were assessed at T0 and T2 with an in-house Interferon Gamma Release Assay (IGRA), consisting of heparin whole blood stimulation with VZV gE peptide libraries (JPT). Each test included a negative (NC) and positive (phytohemagglutinin, PHA) control. IFN-gamma production was measured with the automated ELLA platform. Anti-gE antibody concentration were assessed with a commercial Elisa kit (DBA). Data were presented as median (interquartile range, IQR). Medians were compared using the Wilcoxon rank test.

Results:
We enrolled 25 HE patients (13F/12M, mainly with Non-Hodgkin Lymphoma) and 16 PrID patients (6F/10M, mainly with common variable immunodeficiency or ipogammaglobulinemia). Median age was 67 [59-72) years and 59 [34-65) years, respectively. Median IFN-gamma production after gE stimulation significantly increased after HZ/su vaccination in both HE and PrID subjects (Wilcoxon $p<0.0001$ and $p=0.002$, respectively) (Figure 1A). However, considering as effective a vaccinal response greater than 4-fold increase in IFN-gamma production from T0 to T2 for the gE stimulated condition, 8/25 (32%) HE and 6/16 (38%) PrID patients were considered as non-responder. Concerning B-cell response, a significant increase in anti-gE antibody concentration were observed in both HE and PrID subjects (Wilcoxon $p=0.0004$ and $p=0.0012$, respectively). However, 10/25 (40%) HE and 9/16 (56%) PrID patients showed less than 2-fold increase in anti-gE concentration from T0 to T2.

Conclusions:
VZV gE IGRA is a sensitive and specific tool to assess CMI after HZ/su vaccination in HE and PrID patients. A significant amount of HE and PrID patients had an impaired cell-mediated and humoral response after HZ/su vaccination. The implications of the reduced immunogenicity of HZ/su vaccination in terms of protection from HZ need to be further investigated in wider cohorts of immunocompromised patients with longer observation periods. We can speculate that a different vaccination schedule with supplementary booster doses could be advisable for these subjects. Multcytokine assessment of VZV gE-specific T-lymphocyte responses is currently ongoing in our group, to better characterize CMI in immunocompromised subjects.

344 - Assessment of the specific cellular immune response to the recombinant Herpes Zoster vaccine in people living with HIV

Marco Iannetta *(1)* ***- Grazia Alessio*** *(1)* ***- Livia Benedetti*** *(1)* ***- Luigi Coppola*** *(1)* ***- Anna Chiara Epifani*** *(1)* ***- Giulia Montagnari*** *(1)* ***- Giulia Carla Marchetti*** *(2)* ***- Camilla Tincati*** *(2)* ***- Patrizia Pasculli*** *(3)* ***- Claudio Maria Mastroianni*** *(3)* ***- Loredana Sarmati*** *(1)*

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Assessment of the specific cellular immune response to the recombinant Herpes Zoster vaccine in people living with HIV

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GSK was also provided with the opportunity to review a preliminary version of this abstract for factual accuracy, but the authors are solely responsible for the final content and interpretation.

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Introduction

This study aims to evaluate the specific cell-mediated immunity (CMI) for VZV glycoprotein (g)E in PLWH after receiving the recombinant HZ vaccine.

Material and Methods

Starting from February 2024, two-dose Shingrix regimen 2 months apart was administered to adult PLWH. Blood samples were collected at T0 (before the first dose) and T2 (one month after - second dose). CMI was assessed with an Interferon (IFN)-gamma release assay (IGRA) after overnight stimulation of heparin whole blood with gE peptide libraries (PL)-1 (provided by GSK) and -2 (purchased from JPT Peptide Technologies GmbH). IFN-gamma was measured in supernatant using ELLA. The study was approved by local Ethics Committee (N. 115.23). Data are represented as medians (interquartile ranges). Differences between groups were assessed using the Wilcoxon or the Mann-Whitney test. Correlation was assessed with the Spearman correlation test or the Pearson test. The study is registered in a public clinical study registry (ISRCTN15045512).

Results

A total of 124 PLWH have been enrolled. An interim analysis was conducted analyzing 59 PLWH for whom T2 samples were available (Table 1). A statistically significant increase in IFN-γ production from T0 to T2 was observed after stimulation with gE PL-1 and 2 (Figure 1) ($p<0.001$). At both T0 and T2, IFN-γ production after stimulation with gE PL-1 and -2 were positively correlated (Figure 2) ($p=0.0071$ and $p<0.0001$). At T2, lower IFN-γ production after gE PL-1 and -2 stimulation was observed in PLWH with current CD4 cell count <350 cells/µl and <500 cells/µl and nadir CD4 cell counts <200 cells/µl (Figure 3). Furthermore, IFN-γ production was positively correlated with both nadir and current CD4 counts. (Table 2).

Discussion and Conclusions

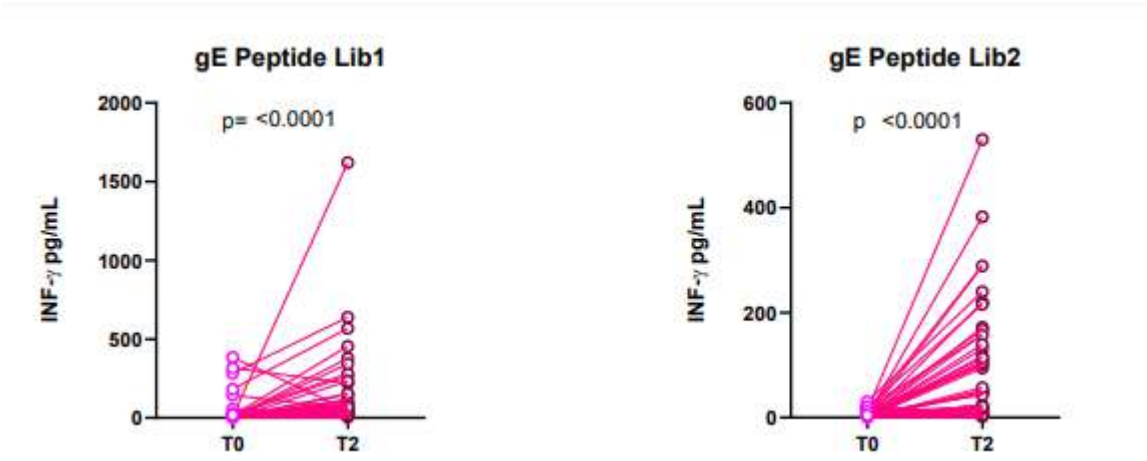
Preliminary data suggest that CMI to HZ recombinant vaccine in PLWH is influenced by patient's current and nadir CD4 count. A previous severe immune impairment, even if current CD4 cell count is recovered, seems to reduce CMI.

Table 1. Characteristics of the cohort, overall

Characteristics		
Total, n (%)		59 (100)
Sex at birth n (%)	Male	49 (83.1)
	Female	10 (16.9)
Nationality, n (%)	Italian	51 (86.4)
	Other	8 (13.6)
Age, median years (IQR)		53 (48-60)
Transmission group, n (%)	MSM	21 (35.6)
	Eterosexual	25 (42.4)
	IDU	6 (10.1)
	Unknown	7 (11.9)
AIDS History, n (%)		22 (37.3)
Nadir CD4 stratum, n (%)	<200 cells/mm^3	18 (30.5)
	≥200 cells/mm^3	26 (44.1)
	Unknown	15 (25.4)
Current CD4, median cell/mm^3 (IQR)		661 (441.8- 950.0)
Current CD4 stratum, n (%)	<350 cells/mm^3	13 (22.0)
	≥350 cells/mm^3	46 (78.0)
	<500 cells/mm^3	23 (39.0)
	≥500 cells/mm^3	36 (61.0)
Current CD8, median cell/mm^3 (IQR)		819 (605.5-984.5)

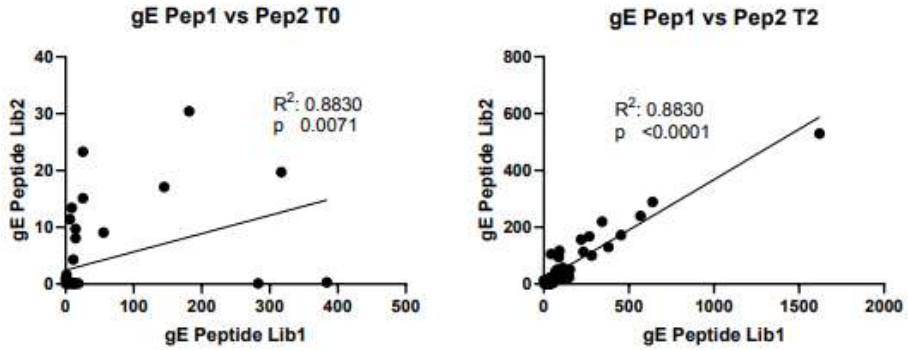
MSM: men who have sex with men; IDU: injecting drug use.

Figure 1. IFN-γ production at T0 and T2 after gE peptide libraries 1 and 2 and IE63 peptide stimulation of heparin whole blood.



A) IFN-γ production increase after gE Peptide Library 1 stimulation at T0 and T2 (2,16 [0,10 – 14,30] vs 53,77 [22,72-148,07], Wilcoxon p<0.0001). B) IFN-γ production increase after gE Peptide Library 2 stimulation at T0 and T2 (0,10 [0,10 – 3,85] vs 21,07 [3,23-114,45] Wilcoxon p <0.0001).

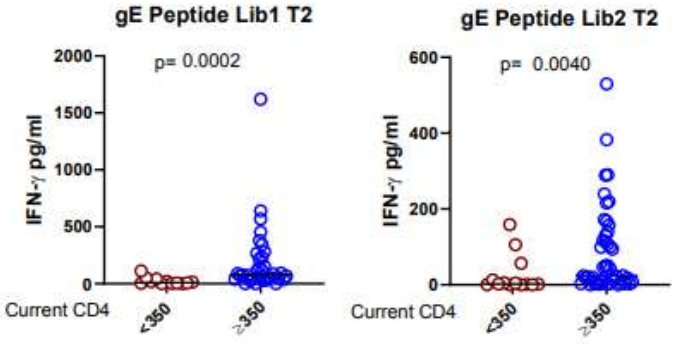
Figure 2. Correlation of IFN-γ production after stimulation of heparin whole blood with peptide libraries 1 and 2 at T0 and T2



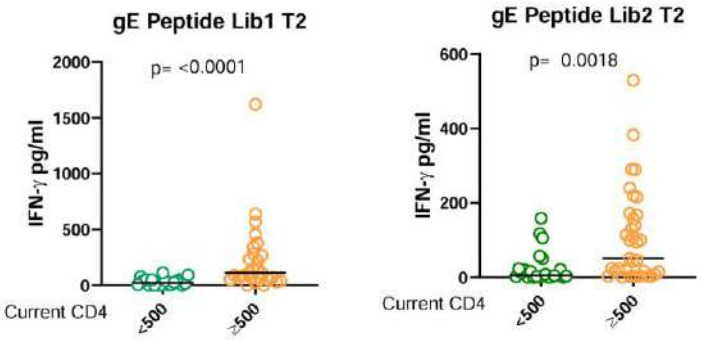
Linear regression between IFN-γ production after gE peptide library 1 and 2 stimulations at T0 (A) and T2 (B). T0: R^2=0.88 and p=0.0071; T2: R^2=0.88 and p< 0.0001

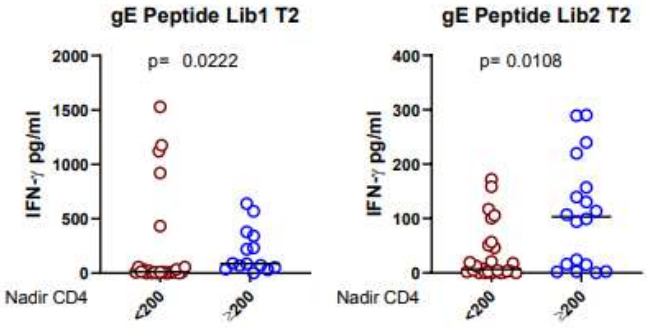
Figure 3. IFN-γ production after stimulation of heparin whole blood with peptide libraries 1 and 2 at T2 after stratification according to current and nadir CD4 cell counts.

A.



B.



C. 

A. Median IFN-γ production after gE Peptide Libraries 1 (A) and 2 (B) stimulation according to current CD4 cell count stratification (cutoff 350 cells/µl) at T2. Peptide library 1, CD4<350 cells/µl: 9,62 [4,02–31,71] vs CD4≥350 cells/µl: 81,03 [46,00-232,60]; Mann-Whitney p< 0.0001). Peptide Library 2, CD4<350 cells/µl: 2,10 [0,10–12,81] vs CD4≥350 cells/µl: 24,28 [7,72-130,36] Mann-Whitney p= 0.0018).

B. Median IFN-γ production after gE Peptide Libraries 1 (A) and 2 (B) stimulation according to current CD4 cell count stratification (cutoff 500 cells/µl) at T2. Peptide library 1, CD4<500 cells/µl: 22,72 [5,24–49,26] vs CD4≥500 cells/µl: 112,00 [53,77-274,57]; Mann-Whitney p<0.0001). Peptide Library 2, CD4<500 cells/µl: 5,08 [1,58–22,04] vs CD4≥500 cells/µl: 50,90 [11,42-162,45] Mann-Whitney p= 0.0018).

B. Median IFN-γ production after gE Peptide Libraries 1 (C) and 2 (D) stimulation according to nadir CD4 cell count stratification (cutoff 200 cells/µl) at T2. Peptide library 1, CD4<200 cells/µl: 11,00 [5,50–55,00] vs CD4≥200 cells/µl: 86,90 [56,94–315,23] Mann-Whitney p=0.0222). Peptide Libraries 2, CD4<200 cells/µl: 6,40 [1,89–49,70] vs CD4≥200 cells/µl: 103,17 [15,37–152,79] Mann-Whitney p=0.0108)

T2	**gE peptide Library 1**		**gE peptide Library 2**	
	Spearman rho	P	Spearman rho	p
Age	0.026	0.862	-0.118	0.378
Nadir CD4	**0.439**	**0.006**	**0.443**	**0.003**
Current CD4	**0.632**	**< .001**	**0.505**	**< .001**
Current CD8	0.242	0.102	**0.313**	**0.017**
CD4/CD8	**0.338**	**0.020**	0.181	0.174
Years living with HIV	**0.336**	**0.021**	**0.267**	**0.044**
Years on ART	**0.308**	**0.039**	0.229	0.095

Table 2. Spearman Correlations between IFN-γ production at T2 and clinical data

T2: one month after second vaccine dose; ART: antiretroviral therapy.

351 - TTV species diversity as a novel biomarker of immune dysregulation in aging

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TTV species diversity as a novel biomarker of immune dysregulation in aging

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Aim of the study: Torquetenovirus (TTV) is a prevalent virus whose clinical significance remains unclear, though it may be linked to aging and immune function. This study examines TTV species, immune impairment, inflammation, and cellular stress response in older adults.

Methods: A subset of RASIG participants from the MARK-AGE study was divided into three groups: Cohort 1A (healthy young adults), Cohort 1B (older adults with mild immune decline), and Cohort 1C (older adults with significant immune impairment). Analyses included TTV load, species diversity, lymphocyte subpopulations, inflammatory markers, and PARP-1 expression/activity as a marker of cellular stress in aging.

Preliminary data: Cohort 1C exhibited significantly higher TTV species diversity and prevalence. Increased TTV species correlated with higher TTV load, elevated CMV IgG levels, and greater immune impairment risk. Specific TTV species were linked to CD4/CD8 ratio changes, and reduced T-cell receptor excision circles, suggesting a potential impact on T-cell homeostasis. TTV viremia positively correlated with CRP and α2-macroglobulin levels, with TTV15 linked to increased α2-macroglobulin levels. PARP-1 showed higher expression and activity in individuals with more TTV species, particularly TTV9 and TTV20.

Conclusions and future prospect : TTV load and species diversity are associated with immune aging, inflammation, and PARP-1 activation suggesting their potential use as biomarkers for immunosenescence and age-related diseases. Longitudinal studies are needed to determine whether TTV species diversity increases due to immune decline or actively contributes to immune dysfunction. While our sample size was sufficient to detect major associations, subgroup analyses (e.g., examining individual TTV species) were limited by low statistical power. Larger studies are necessary to confirm our findings and explore potential confounding factors such as medication use, comorbidities, and lifestyle variables.

353 - A mRNA-based vaccine approach against bacterial diseases: the Streptococcus pneumoniae model

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A mRNA-based vaccine approach against bacterial diseases: the *Streptococcus pneumoniae*

model

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Introduction: mRNA vaccine technology has demonstrated an unprecedented translational potential characterized by high efficacy and safety during the COVID-19 pandemic. In addition to SARS-CoV-2, mRNA platforms also showed strong potential as vaccines against other viral pathogens, such as Zika or influenza virus, but applications against bacterial diseases have been sporadically approached so far. Prevention of diseases caused by *Streptococcus pneumoniae* (Spn) relies on 2 available vaccines: a polysaccharide vaccine (PPV) or protein-conjugated polysaccharide vaccines (PCVs), but several limitations call for improving the strategy for vaccinations against Spn diseases. **Materials and Methods**: We encapsulated within liposomes *in vitro*-synthesized mRNA coding for the proline-rich domain of PspA1 (D39) and PspA2 (TIGR4) and mRNA coding for pneumolysin (Ply) toxoid (PlyT). Mice were immunized and sera were tested to measure antigen specific antibodies by ELISA, and Ply neutralization assay. Groups of female CD1 outbred mice were infected with 10^6 CFU/mouse of the D39 strain of Spn non- opsonized or opsonized with specific anti PspA serum. **Results**: Mice immunized twice with mRNA coding for PspA1 showed a notable specific antibody production in three independent experiments. Mice immunized with mRNA coding for PspA2, on the contrary, did not develop a specific antibody response. However, some mice produced high antibody titers when immunized with the home-made recombinant PspA2 protein. Survival assessment (equivalent to the time of reaching moderate signs) showed significant protection conferred by the specific anti-PspA serum obtained by vaccination with mRNA at 8 µg. **Discussion and Conclusions**: Our preliminary results indicate that mRNA vaccine technology can be exploited not only in the prevention of viral diseases, but also in the fight against bacterial diseases, if the

mRNA codes for an appropriate protein antigen and represent a proof of concept for a mRNA based vaccination tailored for diseases caused by extracellular bacteria and a preclinical phase for a possible future trial.

358 - Probing PON2 function and inhibition with TQ416 analogues: a step toward structural characterization

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Probing PON2 function and inhibition with TQ416 analogues: a step toward structural characterization

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1. Introduction

The human paraoxonase (PON) family includes three highly conserved lactonases. Among them, PON2 exhibits the highest activity against a key acyl-homoserine lactone involved in Gram-negative bacterial quorum sensing. While its role in bacterial defense was long considered primary, emerging evidence shows PON2 also reduces mitochondrial oxidative stress and regulates apoptosis, cell proliferation, and glucose metabolism, highlighting its therapeutic potential. However, the lack of a crystal structure hampers full understanding of the protein binding pocket properties and its mechanism of action. The use of small-molecule probes represents a promising strategy to elucidate PON2 cellular functions. So far, only one inhibitor, TQ416 (a triazolo[4,3-a] quinoline), has been validated. In collaboration with the University of Perugia, a series of fifteen TQ416 analogues was synthesized and tested to determine their half-maximal inhibitory concentrations (IC50), in order to find the best inhibitor.

2. Materials and Methods

Recombinant PON2 (rPON2) was purified from E. coli inclusion bodies using an on-column renaturation protocol, involving Ni-NTA capture of the denatured protein and step-wise urea removal. Monomeric protein was isolated by size-exclusion chromatography and used for activity assays. In contrast, insect cell-derived PON2 (iPON2) was captured from soluble lysates of baculovirus-infected insect cells with a Strep-tactin resin and then further purified with a size-exclusion chromatography. Enzymatic activity was assayed at 37°C using 3-oxo-C12-HSL as a substrate, in a microplate reader, using different concentrations of each TQ416 analogues.

3. Results

Several TQ416 analogues fully inhibited PON2 activity at nanomolar concentrations. A PON2 homology model was developed to aid in data interpretation, revealing two distinct ligand-binding pockets with varying affinities. iPON2 displayed higher solubility and activity than

rPON2, as well as a different oligomerization state. In fact, the purified protein resulted trimeric, with a molecular weight around 130 kDa, this makes it suitable for structural analysis via cryo-electron microscopy. Structure determination is currently ongoing in collaboration with IGBMC, Strasbourg.

4. Discussion and Conclusions

The potent inhibitory effects of the TQ416 analogues and the development of a PON2 homology model both expand the potential of this chemical scaffold and at the same time provides a valuable framework for structure-guided drug design, potentially contributing to the discovery of new ligands. The use of cryo-EM on the more soluble iPON2 may ultimately lead to a high-resolution structure of this elusive enzyme, overcoming the limitations posed by the lack of crystallographic data.

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05 5. Patogeni emergenti e riemergenti

1 - Innovating Toscana virus genomics: a streamlined amplicon-based workflow for whole genome sequencing

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Innovating Toscana virus genomics: a streamlined amplicon-based workflow for whole genome sequencing

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Introduction

Toscana virus (TOSV, Phenuiviridae family, Phlebovirus genus) is a neurotropic sandfly-transmitted virus whose distribution encompasses various countries within the Mediterranean basin. Despite its clinical significance, underreporting and limited availability of complete genomic data hinder a thorough understanding of its genetic diversity and evolution. This study presents a novel amplicon-based whole-genome sequencing (WGS) method using Illumina library preparation kits and proprietary software to optimize workflows and enhance bioinformatic analyses.

Materials and methods

Primers targeting TOSV lineage A S, M and L segments were designed with PrimalScheme on RefSeqs (NC_006319, NC_006320 and NC_006318) to generate 400bp tiled amplicons. Degenerations were introduced at ambiguous sites to enhance sensitivity across phylogenetically diverse strains. Two-pool multiplex library preparation was performed with Illumina Microbial Amplicon Prep (iMAP) and custom primers. De novo assembly was performed with BaseSpace DRAGEN Targeted Microbial software. Sensitivity was assessed on log10 dilutions of viral propagates (10^4 to 10 copies/µl), validation was carried out on propagates (n=7), clinical samples (n=15) and phlebotomine pools (n=5).

Results

45 oligonucleotide primer pairs (26 for segment L, 13 for segment M and 6 for segment S) were generated. Mean % coverage exceeded 90% for viral concentrations as low as 100 copies/µl, dropping to ~70% at 10 copies/µl. All tested propagates yielded ~100% coverage. The correlation between viral load and mean sequencing coverage demonstrated variability for clinical and vector-derived samples, with CSF outperforming both urine and phlebotomine pools, hence suggesting that the matrix type may play a role in sequencing efficacy. This difference likely reflects the less complex nature of CSF as a sample matrix and the higher integrity of the viral genome in CSF samples compared to urine or phlebotomine pools. Overall, the detection limit of this sequencing methodology appeared to fall around a viral load of 20 copies/µl.

Discussion and conclusion

Our study, albeit with preliminary and very promising results for propagates and samples with relatively high Ct values, proposes a streamlined workflow for amplicon-based whole genome sequencing of TOSV. Leveraging commercially available customizable library preparation kits, specifically the Illumina Microbial Amplicon Prep (iMAP), and using Illumina-licensed software for data analysis, the approach offers an efficient, user-friendly method for TOSV genomic characterization, laying the groundwork for broader epidemiological and virological research.

5 - Temporal Shifts in Antimicrobial Resistance among Diabetic Foot Pathogens Before, During and After the COVID-19 Pandemic: A Retrospective Study

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Title:
Temporal Shifts in Antimicrobial Resistance among Diabetic Foot Pathogens Before, During and After the COVID-19 Pandemic: A Retrospective Study

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Abstract
Diabetic foot infections (DFIs) are a major complication of diabetes mellitus, frequently associated with multidrug-resistant (MDR) pathogens. The COVID-19 pandemic, with its disruption of healthcare access and antimicrobial use, may have impacted the microbial landscape of DFIs.

This retrospective study analyzed 1,748 bacterial isolates collected from DFI patients hospitalized at IRCCS MultiMedica (Milan, Italy) across three distinct periods: pre-COVID (2018–2019), COVID (2020–2022), and post-COVID (2022–2024). Microbiological identification and susceptibility testing were performed according to AMCLI and EUCAST guidelines. Statistical evaluation included χ^2 tests, Fisher's exact test, and Z-tests for proportions.

A significant reduction in polymicrobial infections was observed (from 36.7% to 29.1%), alongside a marked decline in methicillin-resistant *Staphylococcus aureus* (MRSA, from 67% to 48%) and carbapenem-resistant *Pseudomonas aeruginosa* (from 25% to 7%). Conversely, a post-pandemic increase in carbapenem-resistant *Klebsiella pneumoniae*(CRE, from 45% to 67%) and the emergence of carbapenem-resistant *Escherichia coli* (13%) were detected. Extended-spectrum β-lactamase (ESBL)-producing *E. coli* also rose sharply from 9% to 46%.

These findings suggest that while infection control measures during the pandemic contributed to reducing certain resistance phenotypes, they may have indirectly fostered the emergence or selection of others. Continuous microbiological surveillance is essential to detect and contain the spread of MDR organisms in chronic infections such as DFIs. These results may support future development of predictive diagnostic tools and targeted antimicrobial strategies in high-risk patient populations.

Keywords: Diabetic foot infections, antimicrobial resistance, MDR, emerging pathogens, surveillance, COVID-19

7 - Use of PMAxx for Assessing Torquetenovirus (TTV) Integrity: Implications for Clinical Evaluations?

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Use of PMAxx for Assessing *Torquetenovirus* (TTV) Integrity: Implications for Clinical Evaluations?

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Introduction: *Torquetenovirus* (TTV) is a widespread, non-pathogenic DNA virus linked to immune status, making it a promising biomarker for immune monitoring. Its load correlates with immune suppression, as observed in people with HIV (PWH) and liver transplant recipients (LTR). However, standard PCR may overestimate viral load by detecting non-intact particles. PMAxx dye, which blocks compromised viral genomes, may improve TTV quantification accuracy. This study investigates PMAxx's potential to enhance clinical relevance in TTV-based immune assessments and decision-making. Specifically, the study aims to evaluate differences in TTV-load between PMAxx-treated and untreated samples, and to assess possible correlations with SARS-CoV-2 IgG antibodies (IgG) and SARS-CoV-2 neutralizing antibodies (nAbs) after SARS-CoV-2 vaccination.

Materials and Methods: This study included three groups: HCV+ individuals (n=10), LTR (n=81), and PWH (n=40). PMAxx treatment (50µM) was applied to 100µL serum and photoactivated for 30 min. TTV-DNA was quantified via digital droplet PCR. *Torquetenominivirus* (TTMV) and

Torquetenomidivirus (TTMDV) were quantified by real-time PCR. IgG antibodies were quantified with Abbott ArchitectR, and values ≥7.1 BAU/mL were classified as vaccine responders. nAbs were assessed using a microneutralization assay with the SARS-CoV-2 Wuhan-D614G strain; titers ≥1:10 were considered positive.

Results: 50μM PMAxx was used to assess TTV integrity in fresh (10 HCV+ samples) and frozen (81 LTR and 40 PWH samples) samples. In HCV+ samples, PMAxx treatment reduced TTV-DNA viremia by 0.66 Log copies/mL ($p=0.0020$). In frozen LTR and PWH samples, PMAxx treatment reduced viremia by 0.83 and 0.55 Log copies/mL, respectively ($p<0.0001$). No significant differences in TTV-DNA across groups were observed. Correlation between untreated TTV-viremia and the difference between treated and untreated TTV-DNA were detected. In a subgroup of samples, similar results were preliminarily observed for TTMV/TTMDV-DNA. Furthermore, we calculated the correlation between Log copies/mL of TTV-DNA and IgG, as well as with nAbs, both in TX patients (TTV-DNA vs IgG: $r=-0.135$; TTV-DNA vs nAbs: $r=-0.129$) and in PWH (TTV-DNA vs IgG: $r=0.002$; TTV-DNA vs nAbs: $r=0.061$). We then checked if these correlations improved when comparing IgG or nAbs with Log copies/mL of PMAxx-treated TTV-DNA, both in LTR patients (treated-TTV-DNA vs IgG: $r=-0.132$; treated-TTV-DNA vs nAbs: $r=-0.161$) and in PWH (treated-TTV-DNA vs IgG: $r=-0.112$; treated-TTV-DNA vs nAbs: $r=0.003$).

Discussion and Conclusions: While treatment with PMAxx efficiently and effectively reduces levels of compromised TTV-DNA, it does not appear to be useful for improving clinical assessment, particularly with regard to the antibody response related to the SARS-CoV-2 vaccination.

13 - Heat Treatment as a Safe-Handling Procedure for Rift Valley Fever Virus

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Heat Treatment as a Safe-Handling Procedure for Rift Valley Fever Virus

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Abstract

Rift Valley Fever virus (RVFV) is a mosquito-borne virus with high pathogenic potential in ruminants and humans. Due to its high potential for spreading, it is considered a priority pathogen, and it is included in the Bluepoint list of the World Health Organization (WHO). Given the high pathogenic potential of the virus, it is crucial to develop a rapid heat-mediated inactivation protocol to create a safer working environment, particularly in medical facilities that lack a biosafety level 3 laboratory required for direct handling of RVFV. Our results reveal the broad tissue tropism of RVFV, showing the virus's capacity for replication in various cell lines. In terms of the thermal stability of RVFV, our findings showed that a 70 °C heat treatment did not fully inactivate the virus within 15 min. However, when exposed to 80 °C and 95 °C, the virus was completely inactivated after 15 min and 5 min, respectively. Additionally, our results indicated that heat-treatment only slightly decreased the integrity of the RVFV genome whether there is a high or low number of viral RNA copies. Overall, the study established a straightforward protocol for heat inactivation that may be beneficial in handling clinical and research samples of RVFV.

22 - A case of Actynomices europeaus

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A case of *Actynomices europeaus*.

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Introduction.
Soft tissues necrotizing fasciitis causes destruction of the skin, subcutaneous tissue, muscles. These infections are usually caused by bacteria such as *Staphylococcus* , *Enterobacterales* and sometimes anaerobes. In literature many cases of polymicrobial infections are described. We describe a case of necrotizing fasciitis due to *Actynomices europeaus*
Material and methods.
A wound abscess exudate from a caucasian male aged 79 arrived in the laboratory in a blood culture bottle. After about 4 days the automated incubator reported significant growth, after that a sample was streaked on plate showing the growth of a facultative anaerobic filamentous gram-positive bacillus which was repeatedly identificated as *Actynomices europeaus* by MALDI TOF mass spectrometer. The manual antibiogram was performed on blood agar plates. The MIC (Minimum Inhibitory Concentration) values of the tested molecules were reported.
Results
The microorganism represented no difficulties to medical treatment, had very low MIC values for amoxicillin(0.016 mg/L), penicillin G and meropenem(0.047 mg/L). Literature data on *Actynomices europeaus* report *in vitro* resistance to metronidazole. The isolated microorganism was susceptible to ciprofloxacin and piperacillin-tazobactam(0.125 mg/L).
Discussion and conclusions.
Empirical treatment of the patient with clindamycin and meropenem reduced the damage of the infection. In our opinion, for such a difficult-to-grow microorganisms, the poor experience of the laboratories could lead to diagnostic neglection as the very few cases of *Actinomyces* infections described in literature seem to show. As always, the networking between infectious disease specialists and microbiologists is a key factor in order to quickly adjust the therapy but this case, clearly shows how a properly trained laboratory allowed the growth and identification of the microorganism. This case also stresses how much there is still to study and improve in order to implement rapid diagnostic tests that include, hopefully, all possible pathogens. Even then, once more, the microbiologist expertise will remain essential to minimize therapeutic failures.

23 - Surveillance of shiga toxin-producing Escherichia coli associated with bloody diarrhea in Northern Italy and coinfections in the molecular diagnostic era.

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Surveillance of shiga toxin-producing *Escherichia coli* associated with bloody diarrhea in Northern Italy and coinfections in the molecular diagnostic era

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Introduction: Infection with Shiga toxin-producing *Escherichia coli* (STEC) is a zoonosis causing gastrointestinal disease with bloody diarrhea in children and adults worldwide. The most severe complication is the haemolytic uraemic syndrome (HUS), a life-threatening thrombotic microangiopathy, that affected 12–23% of STEC-infected paediatric patients. In Northern Italy, a screening program for detecting Shiga toxin in children and their relatives with acute bloody diarrhea has been active since 2010. The screening program involves more than 60 pediatric units (ItalKid-HUS Network) coordinated by Policlinico IRCCS, in Milan.

Materials and Methods: From November 2021 to March 2025, 2,184 fecal samples from children with bloody diarrhea and their relatives were tested. The presence of Shiga toxin genes

was tested by extracting DNA using the STARMag 96 X 4 Universal Cartridge Kit (Seegene Inc., South Korea). The DNA was amplified by a real-time PCR targeting the stx1/stx2 together with 11 other targets of gastrointestinal pathogens (Allplex Gastrointestinal Panel Assays GI and GII-Seegeene, RealStar EHEC PCR Kit 2.0-Altona Diagnostics GmbH, Qiastat-DX Gastrointestinal Panel-Qiagen). Prevalence of STEC and stx1/2 over time, age and rural vs. urban area was defined. Kruskal-Wallis test for continuous variables and Chisquare for trend for categorical variables defined statistical significance results. Data were analyzed by SPSS v.29.0.10.

Results: 115 cases of STEC (5.3%) were diagnosed from 2,184 tested samples between 2021 and 2025, with a median age of positive cases of 5.6 years (interquartile-range, 1.5-13.7 years). The highest number of STEC was diagnosed between the second and third trimesters of the year (71, 61.7%, P=0.01). 52 cases of 115 (45.2%) belonged to rural areas, whereas 42 (36.5%) were from urban areas and 21 (18.3%) from an unknown area. An erratic distribution of positive cases was found over time (P=0.015), with peaks in the third trimester of 2023 (9.4%) and in the second trimester of 2024 (8.7%). 71 cases (53.5%) were tested positive for Stx2, followed by 29 cases tested positive for both Stx1 and Stx2 (25.4%), and 24 cases that tested positive for Stx1 alone (21.1%). No significant differences were detected in the distribution of Stx over time, age, or area (P>0.05). Coinfections were detected in 28 (24.3%) cases. The most prevalent coinfections were Campylobacter (10.4%), detected in 6 stx1, 5 stx2 and 1 stx1/2 STEC, followed by C.difficile ToxB (7.8%) detected in 1 stx1, 7 stx2 and 1 stx1/2 STEC.

Discussion and Conclusions: Our results showed a continuous circulation of STEC over time, regardless of rural or urban area, confirming the importance of the screening strategies and rapid diagnosis of bloody diarrhea. This approach provides the opportunity to early implement therapeutic strategies to prevent or mitigate the severity of the disease in high-risk populations.

30 - FUNGAL METABOLITES SPHAEROPSIDINS A AND B REDUCE FELINE CORONAVIRUS INFECTION IN VITRO

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FUNGAL METABOLITES SPHAEROPSIDINS A AND B REDUCE FELINE CORONAVIRUS INFECTION IN VITRO

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Feline coronavirus (FCoV), an alphacoronavirus, causes mild or chronic subclinical infection in cats. However, genomic mutations can occur in a single infected cat, resulting in changes in cellular tropism of FCoV which acquires the ability to infect macrophages leading to lethal feline infectious peritonitis (FIP). FCoV is closely correlated to both animal and human coronaviruses (CoVs), such as canine coronavirus (CCoV), transmissible gastroenteritis virus (TGEV) of pigs, and CCoV-HuPn-2018 in humans. The known high genomic plasticity of CoVs, and the recent isolation of a novel highly pathogenic FCoV-CCoV recombinant in a FIP outbreak in cats in Cyprus, are focusing scientific research on the development of novel antiviral therapies involving original mechanisms of action to limit the impact of FCoV infection in cats. The aryl hydrocarbon receptor (AhR) is a transcription factor activated by different substrates both endogenous (bilirubin and biliverdin), and exogenous (environmental contaminants and

microbial metabolites) involved in various physiological functions, including microbial defence. During infection, AhR regulates the host response to several CoVs, including FCoV, and in AhR signaling, after ligand binding, the AhR-ligand complex translocated into the nucleus, where it acts with specific DNA sequences to control the expression of target genes encoding the cytochrome P450 enzymes (e.g., CYP1A1 and CYP1B1), then stimulates cytokines release and regulation of the immune response. Due to the growing interest of scientific community for the properties of natural compounds, our interest has focused on fungal secondary metabolites as potential antivirals. Pimarane diterpenes, like sphaeropsidin A (SphA) and its analogue sphaeropsidin B (SphB), compounds with effective antimicrobial, insecticidal and herbicidal activities produced by fungi belonging to the genus Diplodia, are considered promising substances to be used in medicine and agriculture. Hence, in this study, the antiviral activity of SphA and SphB was tested in Crandell Feline Kidney (CRFK) and canine fibrosarcoma (A72) cells infected with enteric FCoV, strain "München". Non-toxic doses of SphA and SphB markedly reduced viral yield and the expression of the viral nucleocapsid N protein during infection in both cell lines. Furthermore, a significant downregulation of AhR and of its downstream target protein CYP1A1 were observed in the presence of both SphA and SphB. Moreover, the treatment with SphA and SphB reactivated both cytoskeletal organization and acidity of lysosomes, which resulted notably affected by infection. In addition, molecular docking studies were performed to investigate the molecular interactions between FCov and SphA-SphB. These computational analyses provided further insights on the mechanism of action of the compounds, supporting the observed antiviral effects *in vitro*. Overall, our findings suggest that both fungal secondary metabolites, by modulating the AhR pathway, exhibit potential antiviral activity to fight FCoV infection.

33 - Antiviral Activity of Isoxazoline-Carbocyclic Monophosphate Nucleotides Against Enterovirus 11: A Promising RdRp Inhibitor

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Antiviral Activity of Isoxazoline-Carbocyclic Monophosphate Nucleotides Against Enterovirus 11: A Promising RdRp Inhibitor

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Introduction: Enterovirus B (EV-B) infections can lead to severe complications, particularly in high-risk pediatric populations. In 2022–2023, several European countries reported clusters of acute hepatitis and liver failure in children associated with a novel Enterovirus 11 (E11) variant. As no targeted antiviral therapies are currently available, there is an urgent need for effective therapeutic options. **Materials and Methods:** Antiviral activity of two isoxazoline-carbocyclic monophosphate nucleotides (4a-4b) was assessed in Vero-76 cells using cytopathic effect (CPE) reduction assays and quantitative RT-PCR. Cytotoxicity was evaluated by MTT assay and microscopic observation after 48 hours of treatment with compound concentrations ranging from 3.2 to 100 µM. **Results:** The results showed that both 4a and 4b nucleotides exhibited certain cytotoxicity when used at concentrations higher than 50 µM. Compound 4a demonstrated strong antiviral efficacy, reducing CPE by 90% and 75% at 25 and 50 µM, respectively, and decreasing viral RNA levels in the supernatant by 72% and 89%. In contrast, compound 4b exhibited only moderate inhibition of CPE (58% and 38%) and did not significantly impact viral RNA levels. A time-course assay further confirmed the inhibitory effect of 4a. The quantification of E11 virus particles produced by infected cells and released into the

supernatant through qRT-PCR showed a progressive reductions in viral RNA by 43% at 10 hours, 65% at 24 hours, and 96% at 48 hours post-infection. **Discussion and Conclusions:** This study provides new insights into the replication kinetics of E11 and highlights the potent antiviral activity of compound 4a. The findings suggest that 4a functions as an RNA-dependent RNA polymerase (RdRp) inhibitor, effectively impairing E11 genome replication and viral progeny production. These results support further development of 4a as a promising antiviral candidate against E11 infections.

57 - Potential of the Peribacillus simplex clade as emerging human pathogens: microbiological and virulence profiling of ten clinical isolates

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Potential of the *Peribacillus simplex* clade as emerging human pathogens: microbiological and virulence profiling of ten clinical isolates

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Introduction: The *Peribacillus simplex* clade (*Ps*c) includes Gram-positive bacteria commonly found in the environment and rhizosphere. However, three recent case reports pointed out *P. simplex* as causative agent of severe human infections. Here, we characterized ten clinical isolates from the *Ps*c for their lifestyle and potential pathogenicity.

Materials and Methods: Identification was performed by MALDI-TOF Mass Spectrometry and 16S rRNA gene sequencing. Growth flexibility was assessed by culturing strains in various conditions and quantifying their sporulation rate. Screening for virulence/pathogenic traits included swimming and swarming motility, biofilm formation, enzymatic activities, presence of *Bacillus cereus* virulence factors-encoding genes, and antibiotic susceptibility. Antimicrobial activity against a panel of bacteria and fungi was tested by the agar overlay assays.

Results: Both identification techniques identified all strains as part of the *Ps*c, but species resolution within the *Peribacillus* genus is challenging. All strains replicated better at 37°C than 25°C, but most growth abilities resulted strictly strain-specific. Strains 2 and 7 displayed the widest range of tolerance to temperature (4-45°C) and pH (3 to 10). Most of the isolates were able to efficiently sporulate. Only strain 4 was able to swim and swarm, whereas the remaining strains were non-flagellated and non-motile. Strain 4 formed biofilm in 24 h, while for other strains (5, 6, and 7) biofilm was produced after 48 hours. All strains showed amylase and gelatinase activities; strains 4 and 6 also secreted proteases. The *cytK* gene was detected in strain 7, indicating its potential secretion of cytotoxin K. Results of the antibiotic susceptibility testing mostly varied between isolates, with strains 3, 4, 5, and 6 displaying higher MIC values and partial resistance profiles (according to EUCAST *Bacillus* spp. breakpoints). All *Ps*c isolates

were active against *B. cereus* and strains 1, 4, 7, 8, and 10 were also active against other Gram-positive bacteria, *Aspergillus brasiliensis*, and *Candida albicans*.

Discussion and Conclusions: The *Ps*c strains investigated herein exhibit very different behaviors and virulence potential. Faster replication at 37°C and sporulation are indicative of good adaptation and dissemination in the environment and clinical settings. Positivity for virulence factors are strictly strain-specific, with strains 4, 6 and 7 showing the highest overall pathogenic potential. The antimicrobial activity demonstrated for some strains could alter the resident microbiota of the host, thus favoring *Ps*c colonization and infection. Our findings support the clinical significance of some *Ps*c strains and confirm them as emerging threats for human health.

59 - Impact of Nirsevimab prophylaxis on RSV bronchiolitis in northeastern Italy

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Impact of Nirsevimab prophylaxis on RSV bronchiolitis in northeastern Italy

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Introduction. The Respiratory Syncytial Virus (RSV) is a leading cause of lower respiratory tract infections in infants worldwide, often resulting in bronchiolitis and significantly impacting thehealthcare system. Due to its seasonal outbreaks and potential severity, particularly in the first months of life, effective prophylactic strategies are needed. During the 2024-2025 winter season, Nirsevimab was introduced for the first time as a prophylactic measure against bronchiolitis in Friuli Venezia Giulia, in northeastern Italy. We investigated the clinical impact of this intervention by analyzing data from the Institute for Maternal and Child Health IRCCS Burlo Garofolo (Trieste, Italy), focusing on the incidence and severity of bronchiolitis during its first year of implementation. **Materials and Methods.** We retrospectively reviewed medical records of all children diagnosed with bronchiolitis at the Institute during the 2024-2025 winter season, comparing them with data from the seven previous seasons. For each hospitalized infant, we collected demographic data, virological results from molecular multiplex testing of nasal

swabs performed with the Respiratory Flow Cell Chip Kit (Vitro Master Diagnostica, Seville, Spain), need for respiratory support, requirement of hospitalization, and its duration. We aimed to assess how the rate of hospitalizations related to bronchiolitis changed following the introduction of Nirsevimab, compared to previous seasons. **Results.** During the 2024-2025 season, 92% of neonates (n = 451) born at the Institute received Nirsevimab prophylaxis. In this period, only 8 infants were admitted for bronchiolitis, a value significantly lower than in previous years (p = 0.02). Among these, 3 infants (37.5%) tested positive for RSV. These findings reflect a marked reduction in RSV-positive cases, along with a sharp decline in hospital admissions and decrease in the need for respiratory support and total hospitalization days. **Discussion and Conclusions.** In this single-center population study, Nirsevimab prophylaxis was widely accepted. This led to a reduction in the burden of bronchiolitis, highlighting the clinical benefits of prevention strategies in pediatric healthcare.

62 - Clonal expansion of K2 OXA-48-producing Klebsiella pneumoniae ST395 in Venice Province

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Clonal expansion of K2 OXA-48-producing *Klebsiella pneumoniae* ST395 in Venice Province

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Introduction. *Klebsiella pneumoniae* ST395 (KpST395; CC258) is an international high-risk clone first reported from an outbreak of OXA-48-producing *K. pneumoniae* (OXA-48-Kp) in France in 2010. Besides *bla*OXA-48, KpST395 was reported to carry *bla*KPC-2, *bla*KPC-3, *bla*NDM-1, and *bla*NDM-1 and *bla*NDM-5 in China, Italy, Russia and Germany, respectively. We described the OXA-48-Kp ST395 clonal expansion in North Eastern Italy. **Materials and methods**. In the period May-July 2024, a total of 57 OXA-48-Kp have been collected from S. Donà di Piave, Jesolo and Portogruaro Hospitals. Species identification, antimicrobial susceptibilities, carbapenemases production and clonal relatedness were ascertained by MALDI-TOF MS, Vitek-2 System, CARBA PLUS (MASTGroup) and PFGE, respectively. Whole-Genome Sequencing (WGS) was carried out through NovaSeq Illumina on seven representative strains. **Results**. All 57 OXA-48-Kp strains retained susceptibility to aminoglycosides only. Three pulsotypes (A-C) were detected by PFGE. The outbreak pusoltype A included 13 sub-types (A-A12): A3 and A9 (n= 8, respectively) were leading patterns, followed by A12 (n=4), A5

(n=3) and A11 (n=2). A3 and A12 led the spread in Portogruaro; A3 circulated in S. Donà di Piave and Jesolo areas as well, but at a lower extent, overcomed by A9 in Jesolo, A9 and A11 in S. Donà di Piave. A9 was missing in Portogruaro. ST395 and capsule K2 were highlited by WGS. The shared resistome consisted in aminoglycoside (*aac(3)-IIa, aac(6')-Ib-cr*), chloramphenicol (*catA1*, *catB4*), beta-lactams (*bla*CTX-M-15) and carbapenems (*bla*OXA-48) genes. SNP-based phylogeny showed relevant genomic relatedness (maximum SNPs difference=77), though clonal identity was obtained between P_16 and P_60 (SNPs=2) only. P_8, collected in 20th May from urine sample in Jesolo community, showed high clonal link (SNPs=8) with P_59, involved in a deadly sepsis in urology ward in Portogruaro on 26th June. P_5, from urine sample of a Portogruaro outpatient on 3rd July was the most genomically distant (SNPs=77), as P_55 (from Jesolo) that exhibited highest similarities with P_16 and P_60 (SNPs=31). The sequenced strains showed genomic distance from other Italian Kp ST395. **Conclusions**. We characterized the alarming emergence and inter-hospital circulation of OXA-48-KpST395 in Eastern Italy.

67 - Antiviral activity evaluation of Tamaricicola sp PN38 and Clonostachys rosea IG119 extracts against Echovirus-11

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Antiviral activity evaluation of *Tamaricicola* sp PN38 and *Clonostachys rosea* IG119 extracts against Echovirus-11

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Introduction: Since July 2022, multiple cases of severe neonatal infections with liver failure have been reported across several European countries, all associated with a novel variant of Echovirus-11 (E11), a member of the Enterovirus genus. Although various molecules have demonstrated promising anti-Enterovirus activity in vitro, only a few have shown efficacy in vivo. Marine fungi, particularly those from underexplored environments, represent a valuable source of bioactive secondary metabolites. *Clonostachys rosea* IG119 and a newly identified strain from the *Tamaricicola* genus (PN38 strain) have previously been characterized for their antimicrobial potential. This study aimed to investigate the antiviral activity of compounds produced by these two marine fungal strains against E11. **Materials and Methods:** Crude extracts from IG119 and PN38 strains were tested for cytotoxicity in Vero-76 cells using the MTT assay and light microscopy, across concentrations ranging from 1.56 to 100 µg/ml. Antiviral activity against E11 was assessed through cytopathic effect (CPE) reduction assays and qRT-PCR quantification of viral RNA in cell culture supernatants following co-treatment with extracts (0.19 to 50 µg/ml). **Results:** Both fungal extracts exhibited cytotoxicity at concentrations above 50 µg/ml. In antiviral assays, IG119 extract showed only weak inhibitory activity. In contrast, PN38 extract demonstrated potent antiviral effects, with a 90% reduction in CPE observed at 3.2 and 6 µg/ml. Corresponding qRT-PCR analysis revealed a significant decrease in viral RNA levels, with reductions of 86% and 94%, respectively, confirming the antiviral potential of PN38 at the molecular level. **Discussion and Conclusions:** These preliminary results highlight the PN38 strain as a promising source of antiviral compounds effective against the emerging variant of Echovirus-11. Further purification and

characterization of the active constituents are warranted to support drug development efforts targeting EV-related infections.

77 - New therapeutic strategies against emerging Arboviruses

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New therapeutic strategies against emerging Arboviruses

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Introduction

Due to the recent rise in outbreaks of flaviviral infections and the lack of vaccines and therapies for most of these viruses, there is an urgent need for effective antivirals. Several approaches are now being explored, including the inhibition of protein-protein interactions and the repurposing of previously licensed drugs, an innovative and a faster drug discovery strategy. In this context, we focused on the interaction between the two flaviviral enzymes, i.e., NS3 and NS5 proteins. NS3-NS5 interaction represents a very appealing target, since it is essential for virus replication and conserved across flaviviruses. In parallel, we also explored use of antifungal azoles' repurposing as a potential treatment against flaviviruses.

Materials and Methods

To identify protein-protein interaction inhibitors, two independent structure-based virtual screenings (SBVS) targeting the binding interface between NS3 and NS5 of dengue virus (DENV), West Nile virus (WNV), and Zika virus (ZIKV) were performed. Starting from the SBVS results, selected hits were tested in an ELISA-based assay to evaluate their ability to inhibit NS3-NS5 interaction. The most promising compounds were tested for their antiviral activity against flaviviruses and for cytotoxicity. The most potent compounds were then characterized through biophysical techniques to demonstrate their binding to NS5. One molecule was also tested in a mouse model of dengue infection. For drug repurposing, some clinically approved antifungals from the azole class were selected to be tested against DENV, WNV, ZIKV, and Usutu virus (USUV). The active antifungals were also assayed in human disease-relevant cells, i.e. JEG-3 and SH-SY5Y cells.

Results

Among the hit compounds selected from the SBVS, some small molecules inhibited the interaction between NS3 and NS5 *in vitro* and the replication of all DENV serotypes as well as ZIKV and WNV and exhibited low propensity to select resistant viruses. The binding of the most potent compounds to viral NS5 protein was demonstrated by NMR and Microscale Thermophoresis (MST). Remarkably, one molecule demonstrated efficacy in a mouse model of dengue by reducing peak viremia, viral load in target organs, and associated tissue pathology. Among the antifungal azoles selected for drug repurposing, some inhibited the flaviviral replication at low micromolar concentrations, without showing cytotoxicity in Vero cells. Moreover, posaconazole and isavuconazole also inhibited virus replication in JEG-3 and SH-SY5Y cells. Regarding the mechanism of action, direct activity on the virion was excluded based on virucidal assays' results. Multiple mechanisms of antiviral activity could be hypothesised from the time-of-addition assays. Further studies are ongoing to elucidate their mechanism of action.

Discussion and Conclusions

Broad-spectrum antivirals are much awaited, and these studies represent a significant advance toward the development of therapeutic molecules with extended anti-flavivirus potential that act by innovative mechanisms and could be used alone or in combination with other antivirals.

84 - INTRAOPERATIVE LAVAGE IN SHOULDER ARTHROPLASTY AS A STRATEGY TO PREVENT PERIPROSTHETIC INFECTIONS

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INTRAOPERATIVE LAVAGE IN SHOULDER ARTHROPLASTY AS A STRATEGY TO PREVENT PERIPROSTHETIC INFECTIONS

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Introduction. Reverse total shoulder arthroplasty (RTSA), increasingly adopted in modern orthopaedic surgery, offers improved pain relief and functional recovery; however, it is associated with various complications, including periprosthetic joint infection (PJI). The most common pathogens implicated in PJI include anaerobic bacteria—primarily *Cutibacterium acnes*—and coagulase-negative staphylococci (CoNS), followed by *Staphylococcus aureus* and Gram-negative bacilli. An emerging approach to reduce the infection risk involves intraoperative lavage with an antiseptic solution, although its efficacy has thus far been demonstrated only in hip and knee arthroplasty. This study aimed to assess the effectiveness of diluted iodopovidone irrigation in reducing bacterial detection during RTSA and to characterize the isolated microorganisms by analysing their virulence factors, with particular attention to antibiotic resistance profiles and biofilm-forming capabilities.
Patients and methods. In a cohort of 65 patients (43 females, 22 males; mean age 74 years; mean BMI 26 kg/m^2), all presenting with multiple comorbidities and undergoing RTSA, three specimens, collected both before and after iodopovidone irrigation, immediately prior to wound closure following prosthetic component placement, were processed for both qualitative and quantitative assessment of bacterial load. For each bacterial isolate, antibiotic susceptibility and biofilm-forming capacity were evaluated.

Results. *C. acnes* was the only anaerobic bacterium identified, while the aerobic flora was predominantly represented by coagulase-negative staphylococci (CoNS); additionally, *S. aureus* and *E. coli* were also detected. Irrigation with povidone-iodine significantly ($p<0.05$) reduced the bacterial load of both *C. acnes* (from approximately 1×10^3 to 3×10^2 CFUs/mL) and CoNS (from approximately 6×10^1 to 3 CFUs/mL). Only 3% of *C. acnes* strains were resistant to clindamycin, and 47% of CoNS exhibited antibiotic resistance, primarily to macrolides and tetracyclines. Antibiotic resistance rates did not show a statistically significant change before and after irrigation. Regarding *C. acnes*, 26.6% of the isolates were biofilm producers, whereas the remaining 73.4% were non-biofilm producers. Among CoNS, 38.1% were biofilm-producing strains while 61.9% were non-biofilm producers. Notably, iodopovidone lavage led to a reduction in biofilm-forming *C. acnes* strains, with no significant effect under aerobic conditions.

Discussion and Conclusions. Intraoperative iodopovidone lavage appears to be an effective measure for reducing bacterial contamination in shoulder arthroplasty, with potential implications for the prevention of PJIs, including those caused by emerging and re-emerging pathogens. Future studies should investigate long-term postoperative outcomes and compare the efficacy of various antiseptic solutions to further optimize infection prevention strategies.

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85 - PS1097 DOWNREGULATES RTN3 AND EXHIBITS POTENT ANTIVIRAL ACTIVITY AGAINST ZIKA VIRUS — ARE THESE EFFECTS MECHANISTICALLY LINKED?

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PS1097 downregulates rtn3 and exhibits potent antiviral activity — are these effects mechanistically linked?

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Introduction

Vector-borne viruses have become a significant global concern due to their rapid geographic spread and impact on public health. Viruses in the Flaviviridae family, such as Zika, Dengue, West Nile, and Usutu, are particularly alarming. Recent outbreaks causing severe complications, like congenital Zika syndrome. Highlights the urgent need for innovative solutions, such as compounds that can target cellular pathways exploited by multiple viruses, offering broad antiviral activity. We recently screened a panel of compounds to identify potential inhibitors of Zika virus replication. Through this screening, we identified PS1097, a highly active compound with exceptional antiviral activity.

Materials and methods

Protein content was analyzed by Western blot. qRT-PCR was used to assess mRNA levels for viral proteins and Reticulon 3 (RTN3), within treated and untreated cells. Lastly, Operetta high

throughput confocal microscopy was employed to assess localization of target proteins inside cells.

Results

PS1097 reduced the levels of RTN3, a protein in the endoplasmic reticulum that plays a role in Zika virus replication. PS1097 treatment decreased RTN3 protein levels, while the amount of other endoplasmic reticulum proteins remained unchanged. Expression analysis by qRT-PCR showed no alteration in RTN3 mRNA levels, indicating that the compound likely acts at a post-transcriptional level. Inhibition of the proteasome did not lead to RTN3 accumulation, suggesting that proteasomal degradation is not involved. A time-of-addition assay followed by western blot analysis revealed that the timing of RTN3 downregulation does not correlate with the onset of antiviral activity. Thus, the two effects may occur through distinct mechanisms. We are currently investigating the roles of autophagy and the unfolded protein response to determine whether these pathways contribute to the reduction of RTN3 and/or the antiviral activity of PS1097.

Discussion and Conclusions

A compound with potent antiviral activity against Zika virus specifically reduces RTN3 protein levels, suggesting an indirect mechanism of action. However, our data indicate that the antiviral activity is not completely accounted for by RTN3 downregulation. Notably, we observed antiviral effects at early stages of infection, prior to any detectable reduction in RTN3 protein levels. These findings imply that additional mechanisms are likely contributing to the antiviral effect of the compound. Our current efforts are focused on identifying these complementary pathways to fully elucidate the compound’s mechanism of action and assess its potential for further development as an antiviral candidate.

87 - Impact of SARS-CoV-2 on respiratory virus prevalence across pre-, during-, and post-pandemic periods

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Impact of SARS-CoV-2 on respiratory virus prevalence across pre-, during-, and post-pandemic periods

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Introduction: The COVID-19 pandemic significantly affected respiratory viruses' circulation, seasonality, and disease burden. This study aimed to assess the impact of SARS-CoV-2 on the frequency of viral respiratory infections at a teaching hospital in Southern Italy, comparing the pre-, during, and post-COVID-19 periods and exploring how the emergence of SARS-CoV-2 influenced the circulation and seasonality of other respiratory viruses. **Materials and Methods**: Between January 2017 and December 2024, 3,930 nasopharyngeal swabs were analyzed using the BioFire® RP2.1 Plus and QIAstat-Dx® Respiratory SARS-CoV-2 Panels. Samples collected before March 15, 2020, were classified as "pre-COVID-19," those taken from March 16, 2020, to May 5, 2023, as "during-COVID-19," and those obtained from May 6, 2023, to December 31, 2024, as "post-COVID-19." Statistical analysis was performed using the

Chi-Square test (p≤0.05). **Results**: Overall, 790 out of 3,930 (20%) patients tested positive for at least one respiratory virus, with a majority being male (473, 60%). Patients ranged in age from 0 days to 98 years (mean age, 60 ± 19 years), and significant positivity rates were observed in the 65-98 age group (p≤0.05) throughout all periods. In the "pre-COVID-19" period, the most prevalent virus was Influenza A (47.5%), followed by Human Rhinovirus (HRV) (19.2%). During COVID-19, SARS-CoV-2 predominated (64.9%), decreasing to 38% post-pandemic, while Influenza A decreased dramatically to 0.9% and increased to 14.2% in the “post-COVID-19” period. Rhinovirus/Enterovirus remained relatively stable (19.2% pre-COVID-19, 20.8% during, 23.2% post-COVID-19). **Discussion and Conclusions**: The distribution of respiratory viruses shifted significantly across departments during and after the pandemic, with SARS-CoV-2 predominantly affecting infectious disease cases. The pandemic notably altered viral co-infection dynamics, with lasting effects into the post-COVID-19 period. Specifically, a marked decrease in Influenza A circulation was observed, while RSV epidemiology remained stable, and significant co-circulation of Rhinovirus/Enterovirus with SARS-CoV-2 persisted. Therefore, since COVID-19 and influenza affect the same high-risk groups, those individuals must be vaccinated against both viruses.

104 - COMPARING DIFFERENT TECHNOLOGIES FOR THE DEVELOPMENT OF A CAPSULAR-BASED VACCINE AGAINST KLEBSIELLA PNEUMONIAE

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COMPARING DIFFERENT TECHNOLOGIES FOR THE DEVELOPMENT OF A CAPSULAR-BASED VACCINE AGAINST *KLEBSIELLA PNEUMONIAE*

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1. Introduction:

Klebsiella pneumoniae is a Gram-negative and anti-microbial resistant bacterium, leading cause of neonatal sepsis in low- and middle-income countries. Despite the urgent need, no licensed vaccines are currently available. Capsular polysaccharides, also known as K-antigens (KAg), are key virulence factors and promising vaccine targets. However, the high diversity of KAg serotypes poses a significant challenge for vaccine development. This study evaluates multiple technology platforms to accelerate the development of a broadly protective, multivalent KAg-based vaccine.

2. Materials and Methods:

A highly prevalent KAg serotype associated with neonatal sepsis was selected as the model antigen.We compared three different approaches: traditional conjugation, nanoparticle-based

delivery, and MAPS technology. For traditional conjugation, an improved 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) chemistry was used and two carrier proteins, CRM_{197} and recombinant tetanus toxoid (8M-TT), were evaluated. In the nanoparticle-based approach, the polysaccharide was presented on nanostructures ranging from 10 to 150 nm, optimizing uptake by antigen-presenting cells and allowing multivalent antigen display. In the MAPS technology, biotin-tagged KAg was linked to rhizavidin-fused proteins, exploiting non-covalent, high-affinity interactions and allowing the combination of KAg with conserved bacterial proteins to potentially elicit broader immune responses. Vaccine candidates were characterized for purity, saccharide-to-protein ratio, and size. Immunogenicity was assessed in mice and rabbits by quantifying antigen-specific antibody titers (total Ig and subclasses), evaluating in vitro bactericidal activity, and analyzing cellular immune responses.

3. Results:

All platforms elicited significant anti-KAg-specific immune responses, with some variability in antibody profiles and functional activity, highlighting the potential of diverse technologies in capsular vaccine development.

4. Discussion and Conclusions:

This study provides valuable insights into the development of a multivalent KAg-based vaccine against *Klebsiella pneumoniae*. The comparative evaluation of these technology platforms in different animal models and looking both at humoral and cellular responses offers data that may inform vaccine design strategies for other encapsulated bacterial pathogens.

Animal studies were ethically reviewed by the local AWB and carried out at a GSK facility in accordance with European legislation EU DIR 63/2010, and the GSK Policies on the Care, Welfare and Treatment of Animals.

105 - Granulicatella adiacens PBPs acylation status through the beta-lactam resistance point of view

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Granulicatella adiacens PBPs acylation status through the beta-lactam resistance point of view

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Introduction: *Granulicatella adiacens* is a Nutritionally Variant Streptococci (NVS), commensal of oral cavity, genital and intestinal tract. It's an opportunistic pathogen that can cause infective endocarditis (IE), particularly in individuals underlying heart disease. Guidelines recommend treatment with penicillin (P)/ampicillin (AMP) or ceftriaxone (CRO) *plus* gentamicin. Therefore, to overcome aminoglycosides nephrotoxicity, the aim of this work is to study the *in vitro* double beta-lactam response evaluating the role of High-Molecular Mass Penicillin-Binding Proteins (HMM PBPs). **Materials and Methods:** beta-lactam antibacterial and synergistic activity and PBPs affinity of *G. adiacens* Is483ll4 BSI clinical isolate were compared to the ATCC 49175 reference strain. PBPs binding was determined by affinity assay exposing cells to BPR, CRO and AMP (alone and in combination), then labelled with a fluorescent reporter (BocillinFL). After visualization by Typhoon FLA 9500, PBPs inhibition

percentages were calculated by ImageJ software. PBPs homologies with *S. pneumoniae* were identified by BLASTp alignment and conserved motifs localized using Clustal Omega. *pbp* genes mutations were detected by genome sequencing. **Results:** The ATCC strain was susceptible to all beta-lactams, while the clinical isolate was not susceptible to P and AMP, resistant to CRO and cefotaxime, and showed higher MICs for BPR and ceftaroline. Combinations of AMP *plus* CRO/ BPR at 1xMIC value were synergistic. The *in silico* analysis of the *G. adiacens* ATCC 49175 genome (acc. N. NZ_CP102283) *via* NCBI identified five HMM-PBPs: 3 transpeptidase/translycosilase enzymes (94, 91 and 80 kDa) and 2 transpeptidases (77 and 66 kDa). *G. adiacens* PBPs showed 1:1 homology with *S. pneumoniae* PBPs (about 39% identity, over 77% coverage). Clustal Omega analysis revealed conserved transpeptidase domain motifs across the 3 species. ImageJ analysis revealed that in both *G. adiacens* strains, 77 and 66 kDa PBPs were never acylated, 91 and 80 kDa were consistently inhibited, while only in the clinical isolate, 94 kDa PBP was acylated by CRO and CRO/AMP combination. **Discussion and Conclusions:** The observed 66 kDa PBP absence of acylation may reflect its homology with low-affinity *S. pneumoniae* PBP2X, a well-established contributor to beta-lactam resistance. The variable inhibition of the 94 kDa PBP likely stems from specific amino acid mutations impacting drug binding. Notably, despite the apparent absence of direct targeting of complementary PBPs, the synergistic effect of dual beta-lactam therapy, potentially through the inhibition of pivotal bifunctional PBPs (91 and 80 kDa), suggests a promising treatment avenue for *G. adiacens* IE that necessitates further investigation to enhance clinical outcomes.

110 - Parvovirus B19 in pregnancy: risk of mother to child transmission and incidence of viral infection in newborns (2012-2024)

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Parvovirus B19 in pregnancy: risk of mother to child transmission and incidence of viral infection in newborns (2012-2024)

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INTRODUCTION: Parvovirus B19 (B19V) is a human pathogenic virus known as the causative agent of fifth disease. During pregnancy, B19V infection is a potential danger for the fetus due to viral ability to cross placental barrier causing severe damages and/or miscarriage. Since early 2024, a marked increase in incidence of B19V infections has been reported in the Metropolitan area of Bologna, as a result of reduced viral circulation after COVID-19 pandemic restriction measures. The impact of this outbreak, particularly among pregnant women and their newborns, is still uncertain. Hence, the present study aims to assess the risk of mother to child transmission in a cohort of patients from 2012 to 2024 with a specific focus 2024 outbreak.
MATERIAL AND METHODS: From 2012 to 2024, 844 pregnant women and 360 newborns (age < 90 days) attending Maternal Fetal Medicine Divisions, Pediatrics and Neonatology Units of hospitals in the Metropolitan area of Bologna were monitored. Samples were tested for specific B19V IgG and
IgM antibodies (LIAISON® Biotrin Parvovirus B19 IgG, IgM - DiaSorin) and/or for the presence of B19V DNA with quantitative real-time PCR (Parvovirus B19 ELITe MGB® Kit - ELITechGroup).
RESULTS: Among 844 pregnant women enrolled in this study, 135 (16%) tested positive for IgM and/or PCR (>1000 copies/mL). Specifically, during 2012-2019 pre-pandemic years, of 444 monitored women, 57 resulted infected with B19V (12.8%), whereas between 2020 and 2022 only 1 infection out of 158 women screened was detected (0.6%). In 2023 an increment in B19V infections during pregnancy was observed for the first time, with 3 B19V positive pregnant women out of 69 women screened (4.3%). Finally, in 2024 an unprecedented increase was reported: among 173 pregnant women screened, 74 resulted infected with B19V (42.8%). Concerning newborns, a total of 360 newborns were screened and 41 tested positive for B19V

DNA (11.4%). Indeed, a marked increase in mother to child transmission incidence was observed. Specifically, while during 2012-2022 years only 19 newborns out of 298 monitored had congenital B19V infection (6.4%, range 0%-12.5%), in 2023-2024 years 22 cases out of 62 monitored newborns were infected with B19V (35.5%). Finally, analysis of B19V viral loads in newborns showed a mean value of 2.78 Log during 2012-2022 years vs. 3.30 Log during 2023-2024 period.

DISCUSSION AND CONCLUSIONS: In order to prevent mother to child B19V transmission, pregnant women need a strict monitoring. Hence, this study highlighted the importance of a correct public awareness on consequences of B19V infection among pregnant women. Moreover, it emphasized the strong need to introduce rational screening procedures to provide proper monitoring and management of complicated pregnancies.

115 - Nasopharyngeal Moraxella spp. and clinical presentation of pediatric respiratory infections in pathogen-negative children

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Nasopharyngeal *Moraxella spp*. and clinical presentation of pediatric respiratory infections in pathogen-negative children

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Introduction *Moraxella catarrhalis* is a common colonizer of the upper respiratory tract in children, and has recently gained attention as a potential contributor to respiratory disease. However, its role is still controversial, with studies suggesting both protective and pathogenic effects. In this study, we investigated how the nasopharyngeal microbiota may influence the clinical presentation of children with respiratory symptoms who tested negative for routine screening for common respiratory pathogens. **Materials and Methods.** This retrospective study was conducted at the Institute for Maternal and Child Health IRCCS Burlo Garofolo (Trieste, Italy) between May 2023 and July 2024. Nasopharyngeal swabs were collected from 51 children under 18 years of age who accessed the pediatric emergency department with respiratory symptoms and tested negative for a molecular panel of respiratory pathogens (Respiratory Flow Cell Chip Kit, Vitro Master Diagnostica, Seville, Spain). Bacterial composition was assessed through 16S rRNA gene sequencing. Patients were divided based on hospitalization status into hospitalized (H_N) and non-hospitalized (NH_N), and stratified by age group. **Results.** The predominant bacterial genera identified were *Moraxella*, *Staphylococcus*, *Streptococcus*, *Haemophilus*, *Corynebacterium*, and *Dolosigranulum*. H_N patients showed higher relative abundance of *Moraxella spp.* (20% vs. 5%) and lower levels of

Streptococcus, Corynebacterium, and *Dolosigranulum* compared to NH_N group. These trends were consistent across all age groups, *Streptococcus spp*. was more common in non-hospitalized children with mild rhinitis, suggesting a possible protective role. A significant association with age was found only for *Corynebacterium spp*. in hospitalized children over 2 years ($p = 0.011$). No correlations were observed between microbial composition, including *Moraxella spp.*, and clinical severity or ventilatory support. **Discussion and Conclusions.** In this cohort of children, differences in nasopharyngeal bacterial composition, particularly involving *Moraxella spp*., were observed between hospitalized and non-hospitalized patients. However, these differences did not correlate with variations in clinical severity. Further research is needed to clarify the role of *Moraxella catarrhalis*, including studies that consider co-infections and host-microbiota interactions.

135 - HIV nucleocapsid proteins as targets for 1,2-benzisothiazol-3(2H)-one benzenesulfonamides

Valeria Manca *(1)* ***- Roberta Loddo*** *(1)* ***- Matteo Incerti*** *(2)* ***- Vanessa Palmas*** *(1)* ***- Marta Cogoni*** *(1)* ***- Rebecca Piras*** *(1)* ***- Luca Virdis*** *(1)* ***- Laura Locci*** *(1)* ***- Aldo Manzin*** *(1)* ***- Giuseppina Sanna*** *(1)*

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HIV nucleocapsid proteins as targets for 1,2-benzisothiazol-3(2H)-one benzenesulfonamides

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Abstract

Since the first cases of AIDS were reported in 1981, more than 88.4 million people have been infected with the human immunodeficiency virus (HIV). Considerable efforts have been made in recent decades to improve access to antiretroviral treatment and prevention programmes, and the number of new infections worldwide has fallen by nearly 60% since its peak in 1995. However, nearly 113 000 HIV diagnoses were reported in the WHO European Region in 2023, an increase of 2.4% over the previous year. Sub-Saharan Africa remains the region most affected by the global HIV/AIDS burden, accounting for more than 69% of all new infections and 14 million AIDS orphans.

In this scenario, we investigated the potential of a new class of small-molecule HIV inhibitors, the 1,2-benzisothiazol-3(2H)-one benzenesulfonamides, which emerged from our previous research on biologically active benzisothiazole derivatives. In cell-based assays, the lead

compound identified, 6, inhibits replication of HIV-1, HIV-2, and HIV-1 variants carrying clinically relevant mutations against non-nucleoside, nucleoside, and protease inhibitors. It has also been evaluated in *ex vivo* models of dendritic cells, macrophages, and T lymphocytes against clinical isolates belonging to different M clades predominant in Africa and other continents. All of the group M viruses tested were moderately inhibited by HS-1, with values ranging from 10 μM to 3 μM. Intriguingly, HS-1 and all reference NNRTIs were very effective ($EC_{99.9}$ < 0.0001 μM) against the NRTI-resistant subtype C (MP1315). They do not interfere with the gp120-CD4 interaction that leads to HIV entry and syncytium formation, nor do they inhibit RT or IN in enzyme assays.

Genome sequencing of HIV-1 mutants selected for resistance to 6 reveals no *pol* or *env* gene mutations. Instead, two mutations are mapped in the *gag* region, which encodes NC proteins involved in early and late key processes of retrovirus replication, suggesting that NC proteins are the target of the title compounds. Compound 6 shows concentration-dependent virucidal activity against cell-free HIV-1 and HIV-2. In conclusion, the class of 1,2-benzisothiazol-3(*2H*)-one benzenesulfonamides shows promise as potential anti-HIV agents due to their structural simplicity, rapid synthetic procedures, and an intriguing mode of action, and deserves further insight aimed at developing new, more potent derivatives.

138 - Triterpenoids from Gymnosporia chevalieri stem and leaves as Inhibitors of Flaviviruses

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Triterpenoids from *Gymnosporia chevalieri* stem and leaves as Inhibitors of Flaviviruses

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Nature's biological diversity is the source of a wide range of bioactive molecules. Natural products, either as pure compounds or as standardised plant extracts, have been successful sources of inspiration for the development of new drugs.

Gymnosporia chevalieri ("*Lõa châu*" Chevalier) is a species endemic to Vietnam. The chemical constituents and biological activities of this species remain relatively new to the scientific community. For the first time, several compounds, pentacyclic triterpenoids, were isolated and characterized from the stem and leaves of *Gymnosporia chevalieri*, including alfa-amyrin and beta-amyrin, beta-amyrenonol, and kaempferitin.

We describe the *in vitro* evaluation of *Gymnosporia chevalieri* extracts and isolated compounds against a broad spectrum of RNA and DNA viruses responsible for important human diseases.

In particular, beta-amyrenonol showed an interesting EC_{50} range of 4-38 µM against two important human pathogens, belonging to *Flaviviridae* family, Zika and Yellow Fever viruses, respectively.

beta-Amyrenonol belongs to a group of naturally occurring compounds called triterpenoids. They are known for their wide range of biological activities and are traditionally used for their anti-inflammatory and antiproliferative properties. Although not directly studied for antiviral activity, beta-amyrenonol may serve as a structural building block, and more specific studies are needed to determine the exact antiviral potential.

139 - Selection of recombinant antibodies for recognizing anti-viral PS-modified Antisense Oligonucleotides

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Selection of recombinant antibodies for recognizing anti-viral PS-modified Antisense Oligonucleotides

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Introduction: Antisense Oligonucleotides (ASOs) modified with phosphorothioate (PS) bonds are a promising class of RNA-targeting drugs for treating viral infections and other diseases. However, their clinical application strongly depends on the development of reliable tools for

monitoring biodistribution and intracellular delivery—critical factors for assessing both treatment efficacy and safety. Despite the growing use of ASOs, analytical methods for its detection in biological systems remain limited. This study addresses this gap by identifying and characterizing recombinant antibody fragments that specifically target PS-modified ASOs, with the aim of creating tools for diagnostic applications and therapeutic monitoring.

Materials and Methods: We selected multiple human single-chain variable fragment (scFv) clones from an unimmunized phage-display library for its ability to recognize with high specificity the PS-modified backbone of ASOs, independently from the nucleotide sequence. Next-generation sequencing (NGS) was used to follow the clones-selection process. Western blot (WB) analysis was employed to confirm the effective intracellular uptake of ASOs and their recognition by the scFv. Additionally, ELISA was used to quantify the internalized ASOs.
Results: The selection led to the identification of several scFv clones with strong binding specificity for the PS backbone, independent from the nucleotide sequence. To better understand the diversity and selection dynamics, NGS was applied to libraries obtained after selection rounds. The resulting data revealed progressive repertoire narrowing and enrichment of a subset of CDR3 sequences. Notably, clone 5E5 demonstrated robust binding activity. WB confirmed that a new PS-modified anti-viral ASO was efficiently internalized by Vero cells and could be reliably detected in cell lysates using scFv-based immunodetection. Furthermore, competitive ELISA assays enabled precise quantification of ASOs within the cells, further validating the specificity and sensitivity of the selected scFvs. These results underscore the utility of scFv-based probes in accurately monitoring the intracellular distribution of ASOs.

Discussion and Conclusions: Our findings support the use of recombinant scFvs as effective tools for the detection and quantification of PS-modified ASOs. The sequence-independent binding of clone 5E5 to the PS backbone makes it a promising candidate for diagnostic assays and therapeutic monitoring. These antibody fragments could enhance quality control, guide dose optimization, and improve safety assessments during the development of ASO-based therapies. Finally, they show the potential to contribute to more effective and personalized treatment strategies for viral infections and other conditions.

145 - Redox homeostasis and cellular antioxidant systems as targets to limit viral replication and the inflammatory response to SARS-CoV-2 and its variants

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Redox homeostasis and cellular antioxidant systems as targets to limit viral replication and the inflammatory response to SARS-CoV-2 and its variants

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Introduction: SARS-CoV-2 has shown rapid genetic evolution, resulting in the emergence of new variants with a different impact on host response. The infection is known to disrupt intracellular redox balance and suppress NRF2, a key regulator of the antioxidant response. In this study, we compared the ancestral Wuhan-1 strain with two variants to define replication

rate, pathways involved in the activation of inflammatory response and redox state changes. In parallel, the antiviral and anti-inflammatory effects of thiol molecules were also evaluated.
Materials and Methods: Human lung epithelial cells (Calu-3) were infected with Wuhan-1 or EG.5.1 or JN.1 strains. Thiol molecules (I-152 and I-152SdAc) were added for 24h or 48h post-infection. Viral replication was assessed by TCID50 assay or by measuring vgRNA intracellular copies by RT-qPCR. Oxidative stress markers and cytokine expression were evaluated by RT-qPCR and the corresponding proteins by western blot. The levels of cytokines were quantified by ELISA assay. Glutathione (GSH) and cysteine were determined by HPLC method.
Results: Viral fitness of the EG.5 and JN.1 lineages was attenuated compared to the ancestral Wuhan-1 strain, with JN.1 exhibiting a significantly slower replication rate. Calu-3 infected with the Wuhan-1, EG.5.1, and JN.1 lineages showed a significant decrease in NRF2 mRNA at 24h p.i. compared to mock condition. Similarly, the downstream Heme Oxygenase-1 (HO-1) and Glucose-6-Phosphate Dehydrogenase (G6PD) mRNAs were markedly reduced at the same time point. A drastic redox change in terms of glutathione (GSH) and cysteine levels was observed at 48h post infection in all the infected samples. The downregulation of NRF2 and G6PD was effectively counteracted by both thiols and the infected and treated samples had comparable or higher GSH levels compared to mocks resulting in significant inhibition of viral replication. All the viral strains triggered high amounts of pro-inflammatory cytokines, conceivably due to the activation of the redox-sensitive transcription factor AP-1. Indeed, viruses, particularly the EG.5.1 and JN.1 strains, induced the phosphorylation of the AP-1 transcription factor c-FOS and a significant increase of [P]-c-Jun. A decrease of [P]-c-Fos and [P]-c-Jun levels was observed in thiol-treated samples, leading to reduced inflammation.
Discussion and Conclusions: Our findings show that impairment of the NRF2 antioxidant pathway is a hallmark of SARS-CoV-2 infection. The AP-1 transcription factor is a potential regulator of inflammation during infection by the Wuhan-1 strain and its variants. Notably, the use of thiol-based reducing agents, by activating NRF2, inhibits both viral replication and the inflammatory response.

148 - Beyond contaminants: Aeromonas spp. in clinically relevant urinary tract infections, a systematic review

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Beyond contaminants: *Aeromonas* spp. in clinically relevant urinary tract infections, a systematic review

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Aeromonas spp. are Gram-negative bacilli capable of causing infections in humans and animals. While gastrointestinal infections are the most common in humans, these bacteria have also emerged as relevant extra-intestinal pathogens, particularly in immunocompromised individuals, where infections may be severe or even fatal. Although often considered a contaminant, *Aeromonas* spp. has been documented as a uropathogen in patients with risk factors, causing clinically relevant urinary tract infections (UTIs). In many cases, isolates showed resistance, highlighting the need for routine antibiotic susceptibility testing (AST) to guide effective therapy.

In line with PRISMA guidelines, a systematic review of the literature was conducted across PubMed, Web of Science, and Cochrane Library, with no language restrictions. From an initial pool of 168 studies, 19 were included in the qualitative analysis. This review aims to characterize the clinical and microbiological features, including risk factors and antimicrobial resistance profiles, of UTIs caused by *Aeromonas* spp.

A total of 19 studies were included: 7 case reports, 8 observational studies, and 4 case series. Patient age ranged from 0 to 93 years. Among the 59 identified patients, sex could not be determined in 9 cases. Of the remaining 50 patients (Pt), 29 were male (58%) and 21 female (42%). Sixty *Aeromonas* spp. strains causing UTIs were identified, including one co-infection with *A. caviae* and *A. veronii*. The most frequent species was *A. hydrophila* (22/60; 36.67%), followed by *A. caviae* (16/60; 26.67%), *A. veronii* (7/60; 11.67%), *A. popoffii*, *A. salmonicida*, and *A. shubertii* (1/60; 1.67% each). In 12 cases (20%), species identification was not possible. The most common risk factors were: urinary catheter/nephrostomy (13 Pt), immunosuppression (10 Pt), diabetes (8 Pt), prostatic hypertrophy/cancer (6 Pt), bladder/urethral diseases (6 Pt), other malignancies (6 Pt), renal stones (4 Pt), liver cirrhosis (4 Pt). Resistance was observed to

fluoroquinolones (5/10; 50%), trimethoprim-sulfamethoxazole (1/8; 12.5%), nitrofurantoin (1/8; 12.5%), third-generation cephalosporins (3/11; 27.27%), carbapenems (1/12; 8.33%), and aminoglycosides (1/13; 7.69%).

UTIs are among the most common bacterial infections and pose a growing public health concern. Environmental pathogens like *Aeromonas* spp., widespread in water and soil, can contaminate water sources and infect both humans and animals. These organisms not only exhibit increasing antimicrobial resistance but also serve as reservoirs of resistance genes through horizontal gene transfer, worsening clinical outcomes and healthcare costs. Detection of high-risk patients and providing targeted therapy are key to antibiotic stewardship within a One Health framework.

149 - Evaluation of the Antiviral Activity of three potential 3C-Like Protease Inhibitors against SARS-CoV-2 KP3.1.1 and Echovirus 11

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Evaluation of the Antiviral Activity of three potential 3C-Like Protease Inhibitors against SARS-CoV-2 KP3.1.1 and Echovirus 11

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Introduction: In the context of the ongoing search for broad-spectrum antiviral agents, protease inhibitors represent a promising class of compounds, particularly against RNA viruses that depend on protease activity for replication. This study evaluated the cytotoxicity and antiviral activity of three potential 3C-Like (3CL) protease inhibitors (11, 193, and 189) against SARS-CoV-2 variant KP3.1.1 and Echovirus 11.

Materials and Methods: The cytotoxicity of molecules 11, 193, and 189 was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay on VERO76 cells exposed to serial dilutions (100-12.5 µM) of each molecule, and cell viability was measured after 48 hours. For antiviral testing, cells were infected with either SARS-CoV-2 KP3.1.1 or Echovirus 11, and treated post-infection with each molecule at 50, 25, and 12.5 µM. Antiviral activity was assessed by cytopathic effect (CPE), spectrophotometric quantification of cell viability, and viral RNA analysis by multiplex real-time PCR, with Cycle threshold (Ct) values used as an inverse indicator of viral load. For Echovirus 11, residual infectivity, was also determined through a $TCID_{50}$ assay on the post-treatment supernatants.

Results: All molecules showed good tolerability in VERO76 cells, maintaining between 65% and 80% cell viability across the tested concentrations. Against SARS-CoV-2 KP3.1.1, spectrophotometric analysis revealed that molecule 11 conferred 67%, 53%, and 40% protection at 50, 25 and 12.5 µM respectively; molecule 189 provided 43%-36% protection, and molecule 193 ranged from 40% to 34% at 50 - 25 µM and at 12.5 µM, respectively. PCR analysis confirmed significant antiviral activity of molecule 11, with Ct values increasing (Ct 32 and 33 at 50 and 25 µM, respectively) compared to the virus control (Ct 13,1). In contrast, molecules 189 and 193 showed lower efficacy (Ct 19–21) across all concentrations. For Echovirus 11, only molecule 11 provided 50% protection at 50 µM and 40% at both 25 and 12.5 µM and decreased the viral titer compared to the initial inoculum (from 10^3 to 10^2 $TCID_{50}$/mL), although the increase in Ct value was modest (Ct 20,3 compared to 16,6 in the virus control).

Discussion and Conclusions: Molecule 11 demonstrated promising antiviral activity against SARS-CoV-2 KP3.1.1, and partial efficacy against Echovirus 11, reducing both CPE and lower viral titers, despite a modest impact on viral RNA levels. These findings suggest the potential of 3CL protease inhibitors, particularly molecule 11, as candidates for further investigation, potentially extending across different RNA viruses.

155 - Temporal and differential expression of Monkeypox virus genome in human epithelial cells

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Temporal and differential expression of Monkeypox virus genome in human epithelial cells

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Introduction The monkeypox virus (MPXV) belongs to the Orthopoxvirus genus. Orthopoxviruses have a linear ~197kb dsDNA genome with a highly conserved central region and two peripheral regions, the first encoding for transcription, replication, and morphogenesis factors, and the second for immune modulating and virulence factors implicated in tropism and pathogenesis. A hierarchical transcription of early, intermediate, and late genes was previously described. The 2022 global outbreak of MPXV clade IIb and the recent upsurge of cases in Central Africa underlined the importance of studying basic biology and viral evolution.

Since 2022, a much higher reproduction number in humans and a predominant role of sexual transmission have been observed. The temporal expression definition of orthopoxvirus genomes, mainly based on vaccinia virus studies, is challenging due to the high number of genes, close timing of expression, and readthroughs of transcripts.

Material and Methods Human vaginal (VK-2/E6E7), ectocervical (Ect-1/E6E7), intestinal (Caco-2), and renal (HK-2) epithelial cells were infected with MPXV clade IIb (MOI 0.1), infectious virus release was measured, and whole viral transcriptomic profiling was performed by RNA-seq.

Results Hierarchical cluster analysis of infected VK2/E6E7 cells identified two temporal groups: a) 64 genes highly expressed at 2 hpi (cluster 1); b) 115 genes highly expressed at 24-48 hpi (cluster 2). In cluster 1, MPXV genes were mainly located in terminal regions, and their functions were associated with viral entry, transport to replication sites, immune evasion, and DNA replication/transcription. Genes in cluster 2, mainly in the central region, were associated with DNA replication/transcription, and virion morphogenesis. Differential expression of MPXV genes was observed at 48hpi in epithelial cells derived from different anatomical districts. VK2/E6E7 and Caco-2 exhibited a higher percentage of viral reads and higher production of infectious particles (28.6% and 16.4%; 3.2 and 3.8 Log $TCID_{50}$/mL). Ect1/E6E7 (10.3%; 2.7) and HK-2 (3.5%; 2.0) showed lower percentages of viral reads and released infectious particles. The viral transcriptome analysis revealed two profiles: (i) 84 genes highly expressed in Ect1/E6E7 and HK-2 cells, with a high abundance of genes associated to “host-virus interaction” (ii) 95 genes highly expressed in VK2/E6E7 and Caco-2 strongly associated with “transcription and RNA processing”, and “virion structure and morphogenesis” functions.

Discussion and conclusions The analysis of the temporal and differential transcriptome of MPXV helps in comprehending its basic biology, evolution, and interactions with the host. The differential expression of viral immune-modulating factors may explain differences in viral tropism and spread.

156 - Increase of urinary tract infection in non-hospitalized patients: a three-years evaluation

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Increase of urinary tract infection in non-hospitalized patients: a three-year evaluation

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INTRODUCTION: Urinary tract infections represent the most common bacterial infections that people get both in hospitals and in community settings. Urinary tract infection symptoms are the second most common reason to prescribe antibiotics, and that frequently result in consultations in primary care, where the majority of suspected cases are addressed. Simple lower urinary tract infections often go away quickly, and many of them are self-limiting. The prevalence of UTI increases with age, being the prevalence in women over 65 years approximately twice that of the general female population. For women who are younger, more frequent sexual encounters significantly raise the risk of urinary tract infections, and it's typical for them to return within half a year. European studies show that repeated infections correlate with more time off work and more doctor appointments. Furthermore, women with recurring urinary tract infections experience a decline in their quality of life. UTI is diagnosed using a combination of urinary symptoms and urine culture, which shows numbers of a known uropathogen above a given threshold (usually defined as >1000 cfu/ml of urine, but thresholds as ≤ 100 cfu/ml and ≥ 100000 cfu/ml). Many bacteria are frequently present in the urins of healthy individuals without any symptoms. The purpose of this study is to examine the epidemiology of non-hospitalized patients and to track antibiotics resistance activity.

MATERIALS AND METHODS: Clean catch mid-stream urine specimens were collected from all subjects. Uropathogens were isolated and identified by using conventional standard techniques: samples were cultured on Blood agar, MacConkey agar and Sabouraud Dextrose agar. Identification and antibiotic susceptibility pattern was determined on VITEK 2 (Biomereux®) and confirmed on Mueller-Hinton using Kirby-Bauer disc diffusion method.

RESULTS: In the period between January 2022 and December 2024, using culture based methods, 4466 patients have been analysed in microbiology laboratory of HICC: 60% were negative and 40% positive; antibiotic resistance occurred in 227 samples, 5%.

DISCUSSION AND CONCLUSION:

Despite UTI are very common, there is a significant lack of data regarding accurate incidence; in particular, in non-hospitalized people. The financial implications of UTI are enormous. In our study the antibiotic resistances are 5% totally (3.5% ESBL, 0.8% VAN+ and 0.7% KPC). The resistance pattern of organisms from breakthrough UTIs is related to the type of antibiotic prophylaxis that has been prescribed. There is a growing need to ensure appropriate therapy with agents that maximize success for community-acquired infection while minimizing the risk of the development of antimicrobial resistance. Our commitment focuses on patient-centered outcomes, throught continue surveillance, family doctors stewardship in terms of direct and indirect costs, such as those related to the antimicrobial resistance and hospitalization.

167 - Mycobacterium tuberculosis Complex MDR Strains in the Calabria Region, Southern Italy: a Retrospective study.

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Mycobacterium tuberculosis Complex **MDR Strains in the Calabria Region, Southern Italy: a Retrospective study.**

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Introduction Tuberculosis (TB) remains a global health challenge. Migration from high-incidence regions contributes to increasing cases and the spread of rifampicin-resistant (RR) and multidrug-resistant (MDR) strains. Herein, we evaluate the burden of infection in the Calabria Region, additionally the RR and MDR *Mycobacterium tuberculosis* (MtC) strains were characterized by diagnostic routine and Next-Generation Sequencing (NGS). **Materials and Methods** Between 2022 and 2023, respiratory samples were collected from patients attending to University Hospital, Regional Tuberculosis Center. Microscopic examination with Kinyoun/fluorochrome staining, bacterial isolation in culture on Lowenstein-Jensen and Middlebrook 7H9, MtC detection and *rpoB*, *katG*/*inhA* mutations by real-time PCR were performed. MPT64 antigen was detected by immunoassay. MtC identification, drug resistance mutations were tested by Line Probe Assay. Phenotypic Drug Sensitivity Test examined susceptibility to first- and second-line drugs. Single multiplex PCR was performed to amplify 18 drug resistance genes. Sequencing was performed by MiSeq Illumina® platform. Sequencing data were uploaded to a secure web app for automated analysis. **Results** Overall, 101 MtC strains were isolated from biological specimens. In 2022, 47% (47/101) of cases were recorded, while in 2023, 53% (54/101). Among our patients, the 69/101 were male and the median age was 42 years old (ranging from 3 to 93). Foreign citizens represented the 66% of the total cases (67/101), including people from Romania (20.8%), Morocco (11.9%), Gambia (7.9%), and other countries (26%). A total of 85% strains (86/101) were susceptible to the first-line drugs, while the 15% strains (15/101) showed resistance, primarily to pyrazinamide (5/15), isoniazid (4/15), and rifampicin (RR, 2/15). Interestingly, the 13% (2/15) was resistant to the combination of isoniazid, rifampicin and ethambutol (MDR). Resistance to the combination of isoniazid and

rifampicin (MDR) was observed in 1/15 of cases, additionally 1/15 displayed resistance to the combination of isoniazid and pyrazinamide. By sequencing analysis, specific mutations associated with drug resistance in the *rpoB* (S450L, H445N)*, katG* (S315T)*, embB* (Y319C, M306V), *pncA* (G17D)*, rpsl* (K43R)*, gid* (S136_)*, eis* (c-10t, g-12a) and *ethA* (Y140_) genes were found in 3 MDR strains. **Conclusions:** This study showed the TB spread in the Calabria Region, particularly among foreign patients and especially males. The migration into Southern Italy is a recent and growing phenomenon. The 15% of resistant MtC strains highlights the need for microbiological surveillance to promptly detect resistance. Prevention, early diagnosis, and targeted therapies can limit drug-resistant TB and enhance patient outcomes.

180 - Emergence of Multidrug-Resistant Streptococcus agalactiae in late pregnancy: Virulence, resistance patterns, and interaction with protective Lactobacilli

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Emergence of Multidrug-Resistant *Streptococcus agalactiae* in late pregnancy: Virulence, resistance patterns, and interaction with protective Lactobacilli

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Introduction. *Streptococcus agalactiae* (Group B Streptococcus, GBS) is a gram-positive bacterium that colonizes the human gastrointestinal and genitourinary tracts that represents the leading cause of severe neonatal infections. Maternal transmission can result in early- and late-onset disease, often leading to invasive infections, long-term sequelae, or fatalities. GBS is also an opportunistic pathogen in the elderly and immunocompromised. The rise of antibiotic-resistant strains complicates prevention and treatment strategies. This study aims at investigating antimicrobial resistance patterns, virulence gene profiles, and interactions with protective *Lactobacillus* species in GBS strains isolated during late pregnancy screening. Materials and Methods. Thirty-one clinical GBS isolates were collected from private healthcare facilities in Rome, mainly from vaginal swabs (93%), with few isolates from urine, and feces. Hemolytic activity was tested on blood agar; antimicrobial susceptibility was assessed with the VITEK system; virulence genes were detected by monoplex PCR; and interactions with

Lactobacilli were evaluated through co-culture assays. Results. Resistance rates were 77% for tetracycline, 73% for rifampicin, 40% for erythromycin, and 20% for clindamycin. All strains remained susceptible to penicillin, linezolid, moxifloxacin, teicoplanin, tigecycline, trimethoprim/sulfamethoxazole, and vancomycin. Moderate susceptibility to levofloxacin was observed in all but one strain, raising concerns for future resistance. Notably, 33% of isolates displaying a multidrug-resistant (MDR) phenotype were collected during the last weeks of pregnancy. Molecular analysis showed a high prevalence of virulence genes. Adhesion genes (*fbsA*, *fbsB*, *lmb*) were present in 77–100% of isolates. Hemolysis genes (*cylB*, *hylB*) were detected in 97%, while immune evasion genes (*scpB*, *bac*, *bca*) appeared in up to 58%. The capsular protein gene (*rib*) was found in 50% of cases. Discussion and Conclusions. These results highlight the clinical relevance of monitoring GBS colonization in late pregnancy, especially with the emergence of MDR strains carrying conserved virulence profiles. The prevalence of adhesion and hemolytic factors suggests their key role in perinatal transmission. Ongoing studies are examining GBS serotypes and the inhibitory potential of *Lactobacilli* through competition assays, thereby promising microbiota-based strategies for reducing GBS colonization and transmission risk.

186 - SARS-CoV-2 infection induces changes in host alternative splicing patterns in bronchial epithelial cells

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SARS-CoV-2 infection induces changes in host alternative splicing patterns in bronchial epithelial cells

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Introduction. SARS-CoV-2 has been shown to manipulate the host splicing machinery, influencing the expression of genes involved in immune responses. Recent studies indicate that alternative splicing (AS) plays a crucial role in SARS-CoV-2 pathophysiology, with global alterations in AS patterns correlating with disease severity. Specific AS transcripts, including those of genes like CD74, LRRFIP1, and OAS1, have been linked to reduced antiviral immunity. Viral proteins may also interact with cellular splicing-related (SR) proteins, affecting viral gene expression. Since viral infection can influence cellular splicing machinery, altering both viral and host cell gene expression, we aimed to analyze the impact of SARS-CoV-2 on AS.
Materials and Methods. In this study, we investigated differential splice variant expressions in transcriptomic data from CFBE41o$^-$ WT cells infected with SARS-CoV-2 at 48 hours post-infection, compared to uninfected controls. Using the DEXSeq tool, we analyzed differential expression of splice variants within our data. Additionally, Gene Ontology (GO) functional enrichment analysis was performed on genes with differential splice variant expressions. We also focused on genes with significantly deregulated exons to assess whether the resulting isoforms were coding or non-coding.
Results. We identified 5,750 genes with significant changes in splice variant expression, enriched in pathways related to mRNA processing, RNA splicing, and ribonucleoprotein complex biogenesis. SARS-CoV-2 infection was associated with increased production of non-coding isoforms in genes involved in interferon signaling (e.g., STAT1, OAS3, IRF6) and MHC class I antigen presentation (e.g., PSMB3, HLA-F), suggesting viral suppression of antiviral responses. Several SR proteins and RNA-binding splicing regulators (SRSF3, U2AF1, RBM5, RBM39), essential in the host response to viral infection and inflammation, showed altered exon usage favoring unproductive isoforms, indicating widespread splicing deregulation.

Discussion and Conclusion. These findings align with previous reports of viral manipulation of host splicing machinery and suggest that SARS-CoV-2 may promote intron retention, nonsense-mediated decay (NMD), and the production of CDS-undefined isoforms to dampen host immunity and modulate splicing homeostasis. Our results support a model where SARS-CoV-2 orchestrates a splicing-based strategy to suppress host defense by inducing non-coding isoforms of key antiviral and splicing-related genes. Future studies will explore the functional implications of these isoform shifts on viral replication and host cell physiology.

191 - PBP1A and LdtJ support cell envelope homeostasis and impact selection of Colistin-resistant LOS-deficient variants in Acinetobacter baumannii

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PBP1A and LdtJ support cell envelope homeostasis and impact selection of Colistin-resistant LOS-deficient variants in *Acinetobacter baumannii*

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1. Introduction: *Acinetobacter baumannii* is a nosocomial and community acquired Gram-negative ESKAPE pathogen, often multi- or pan-resistant to antibiotics. Its multilayered cell envelope consists of an inner symmetric membrane, a thin layer of peptidoglycan (PG) and an asymmetric outer membrane (OM), enriched in lipooligosaccharide (LOS) on the surface-exposed leaflet, conferring the barrier properties to the cell envelope. Colistin targets the lipid A domain of the LOS and is one of the last-line resource for multidrug-resistant (MDR) *A. baumannii* infections. Despite lipid A being essential for most Gram-negative bacteria, colistin-resistant (Col-R) LOS-deficient (LOS-) *A. baumannii* was reported to be isolated after colistin exposure in strains producing low amounts of the penicillin-binding protein 1A (PBP1A) or in Δ*pbp1A* mutants. PBP1A is a class A bifunctional transglycosylase and $_{DD}$-transpeptidase, that synthesizes 4-3 cross-links of the PG, with a role during cell division. In this study, we investigated the interplay between PBP1A and the $_{LD}$-transpeptidase LdtJ, the only enzyme that synthesizes the 3-3 cross-links of the PG in *A. baumannii*. **2. Materials and Methods**: The single Δ*pbp1A* and Δ*ldtJ* and the double Δ*ldtJ*/Δ*pbp1A* mutants of the MDR strain ABUW 5075 and the susceptible laboratory strain ATCC 17978 were characterized for growth, viability, morphology, and PG composition by high-performance liquid chromatography in comparison with the WT. The broth microdilution method and SDS/EDTA sensitivity assays were performed to reveal

defects in the cell envelope. The molecular interactions between LdtJ and PBP1A were assessed by a pull-down assay and confirmed by mass spectrometry. Col-R LOS- variants were isolated and characterized for growth and morphology with or without colistin. Susceptibility to other antibiotics was evaluated by E-tests. **3. Results:** In both *A. baumannii* genetic backgrounds, Δ*ldtJ*/Δ*pbp1a* mutants showed additive defects in cell growth, viability and morphology compared to the wild type and single mutants. Δ*ldtJ*/Δ*pbp1a* mutants also showed severe defects in OM permeability and integrity. The functional interplay between LdtJ and PBP1A was supported by co-immunoprecipitation that demonstrated a molecular interaction between them. Notably, Col-R LOS- colonies were recovered at a higher frequency in the Δ*ldtJ*/Δ*pbp1a* mutants with respect to the wild type and the single mutants. These variants displayed severe morphological defects and, as a trade-off, became susceptible to vancomycin, bacitracin and daptomycin. **4. Discussion and Conclusion:** The results show that LdtJ and its 3-3-crosslinks, albeit not strictly essential, are crucial to support cell survival in the absence of PBP1A and that LdtJ is not required to develop colistin resistance even when PBP1A is inactive. The ability to generate Col-R LOS-deficient variants at higher frequency in the *Δpbp1a* and *Δpbp1a*/Δ*ldtJ* mutants suggests that, even if LOS can be conditionally dispensable in *A. baumannii*, this likely requires PG synthesis and remodeling to be re-programmed. Overall, the results highlight the intimate connection between the PG and the OM and its implications for cell physiology and antibiotic resistance, highlighting the remarkable adaptability of *A. baumannii* to extreme challenges.

196 - Unraveling cell type-specific tropism of respiratory viruses and oxidative stress-related inflammation in Air-liquid interface culture of respiratory epithelium

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Unraveling cell type-specific tropism of respiratory viruses and oxidative stress-related inflammation in Air-liquid interface culture of respiratory epithelium

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Introduction. Respiratory viruses can interfere with the Nrf2 pathway, which is activated to cope with oxidative stress and regulates the inflammatory response. Although several studies have investigated the role of redox alterations in the progression of viral infections, the specific contribution of different airway cell types to redox imbalance remains poorly characterized. Therefore, the aim of the project is to exploit innovative in vitro systems that can recapitulate the heterogeneous microenvironment of the airway epithelium and to correlate the cell type-specific tropism of different respiratory viruses with alterations of redox state and inflammatory response, deepening the contribution of the cell types in the development of severe respiratory infections.
Materials and Methods. Normal Human Bronchial Epithelial (NHBE) cells were grown under Air Liquid Interface (ALI) for 21 days and infected with human or avian influenza A virus (IAV) strains or seasonal coronavirus (HCoV). At different timepoints post infection (p.i.), the Nrf2-related antioxidant factors and the cytokines were analyzed by measuring mRNA and protein levels by RT-qPCR and western blot. A co-immunostaining of a cell-type marker and a viral protein was performed to evaluate the tropism of IAV and HCoV in the bronchial epithelium.
Results. All the viral strains used in this study showed a peak of viral replication 24h p.i. in ALI cultures. The avian IAV strain replicated more efficiently compared to human H1N1 and HCoV, inducing stronger inflammation and greater damage to the epithelial barrier; this was consistent with an increased transcription of cytokines and other inflammatory genes. At the

same timepoint, a decrease of Nrf2 mRNA was observed during IAV infections. At later time points, we observed, for both IAV strains, a decreased protein levels of Nrf2, and some Nrf2-regulated genes. In HCoV infection this modulation was evident only for protein expression, pointing to post-transcriptional mechanisms of inhibition. In addition, preliminary immunofluorescent experiments suggested that both IAV strains and HCoV have a preferential tropism for ciliated cells, at least at later stages of infection.

Discussion. The data points to a strong connection between redox state and inflammation, and since we observed a preferential tropism of IAV and HCoV for acetylated tubulin-positive cells, these alterations could mainly involve ciliated cells. Further experiments will clarify if the redox and inflammatory status of non-infected cells can be influenced by the presence of neighboring infected cells. Also, we plan to better characterize the cellular tropism of isolated respiratory viruses in association with redox state changes, in bronchial, nasal, and alveolar ALI models.

202 - In vitro modeling to study West Nile virus neuropathogenesis, utilizing human induced pluripotent stem cell-derived human brain organoids (hiPSC-HBOs)

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***In vitro* modeling to study West Nile virus neuropathogenesis, utilizing human induced pluripotent stem cell-derived human brain organoids (hiPSC-HBOs).**

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Background and Aims: West Nile Virus (WNV), a mosquito-borne flavivirus, has emerged as a significant public health threat in Europe, with recent years seeing an increasing cases of West Nile neuroinvasive disease (WNND) cases. Traditional virus-host interaction studies using animal models and 2D cell cultures have limitations in terms of ethical concerns and physiological relevance. To address this, hiPSC-HBOs were utilized to model WNV-induced neurological damage and neuroimmune responses.
Methods: Organoids were matured to day 42 before infection with WNV B9560, previously propagated and titrated by plaque assay in Vero cells. Infections were conducted using three Multiplicity of Infection (MOI) for 24 hours. Supernatants were collected at 24, 48 and 72 hours, organoids were either fixed in cold PFA for cryosectioning and immunofluorescence or lysed in RLT buffer for RNA extraction. WNV RNA in supernatants (SN) and organoids was quantified by RT-qPCR. Infectious virions were confirmed by plaque assay, with results expressed as PFU/mL and relative replication rates. Neuroimmune responses were assessed by ELISA (SN) and SYBR Green RT-PCR (RNA transcripts). Viral tropism will be examined by immunohistochemistry using anti-WNV envelope protein antibodies and confocal microscopy, with co-staining to identify infected neurons, progenitors, astrocytes and glial cells. Additionally, RNA-seq for global expression, and spatial transcriptomics to map infection across brain regions will be performed.
Preliminary data: Brain organoids supported productive WNV infection, with viral RNA detectable as early as 24 hpi and peaking at 72 hpi. RT-qPCR revealed a dose-dependent increase in viral genome copies in both organoid lysates and supernatants. At low MOI, viral load increased over time, reaching 10^5, 10^6, and 10^7 copies/mL at 24, 48, and 72 hours, respectively. In contrast, high MOI maintained consistently elevated levels. Despite infection, no structural changes or cellular debris were observed in organoids by microscopy. Plaque assays confirmed active replication, with peak titers of ~10^6 PFU/mL under high-dose

conditions. RT-qPCR neuroimmune profiling showed elevated expression of IL-6(1x), IL-10(2x), CXCL5(4x), TNF-alpha(1x), IFN-alpha(1x), and IFN-beta(2x) compared to uninfected controls (x), while IFN-γ was undetectable. No cytokines were detected in SN by ELISA.
Future Perspective: Immunofluorescence analysis will be conducted to investigate the expression patterns of WNV in hiPSC-HBOs. Collectively, these studies are expected to establish human brain organoids as a robust in vitro model for elucidating WNV neuropathogenesis, viral replication dynamics, cellular specificity, and associated host immune responses.

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207 - Emerging and re-emerging paramyxoviruses: inhibitory peptides targeting the envelope glycoproteins

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Emerging and re-emerging paramyxoviruses: inhibitory peptides targeting the envelope glycoproteins

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Introduction: Globalization and human encroachment into native wild-life habitats will likely continue to increase the number of emerging and re-emerging zoonotic viral diseases. In recent years, there has been a surge in the identification of widely circulating paramyxoviruses, particularly those hosted by several animal reservoirs. Among these, the genera Henipavirus, Morbillivirus and Pararubulavirus are of particular concern. Peste des petits ruminants virus (PPRV) is classified as a small ruminant morbillivirus and shares a close antigenic relationship with other viruses of the same genus, including rinderpest virus (RPV), measles virus (MV) and canine distemper viruses (CDV). These morbilliviruses have the propensity to cross species barriers, which highlights their potential towards inter-species transmission and novel host adaptation. In the same way, zoonotic Pararubulaviruses, as Menangle (MenV) and Sosuga (SOSV) viruses, cause spillover events and could induce mild symptoms or remain asymptomatic in the human host. In this study, we focused on the paramyxovirus mechanism of entry to elucidate their pathogenicity. The ability of paramyxoviral glycoproteins to merge viral and host membranes allows entry of the viral genome into host cells, as well as cell–cell fusion, an important contributor to disease progression. Viral fusion acts through a common mechanism, the formation of a complex between two heptad repeat (HR) regions in the fusion (F) glycoproteins. The HR regions N and C terminals (HRN, HRC) are initially separated in an intermediate termed "prehairpin", which bridges the viral and cell membranes, and then fold

onto each other to form a 6-helical bundle (6HB), driving the two membranes to fuse. Molecular and structural advances in our understanding of the paramyxovirus membrane fusion machinery gave rise to various therapeutic approaches aiming at inhibiting viral infection, including the use of glycoprotein-mimic peptides. **Materials and Methods:** Here, we describe the design, synthesis, and evaluation of HRC peptides derived from the consensus domain of F glycoproteins. The platform we present here for assessing the peptide antiviral potency relies on functional assays that mimic the viral entry into a biosafety level (BSL)-2. Additionally, we had proven peptides inhibitory activity in live virus neutralization assay in vitro, using surrogate viruses of the same genus, which can be easily managed in BSL-2 facilities. **Results:** HRC-derived peptides effectively impaired the fusion process, with a half-maximal inhibitory concentration (IC50) of approximately 15 μM for all viruses tested, representing encouraging results given that these peptides are crude preparations. Similar results have been recorded in the live virus entry assay. **Discussion and conclusions:** We hypothesize that HRC-derived peptides are likely to act in a dominant-negative manner by binding to the F HRN, thereby disrupting the 6HB. We are also exploring synergistic potential by combining consensus peptides with well-known paramyxoviral inhibitors as zanamivir, the N-(3-cyanophenyl)-2-phenylacetamide, or the fusion inhibitory peptides Z-d-Phe-l-Phe-Gly (FIP). In conclusion, we are optimizing our crude preparation by considering standard modifications, such as dimerization and lipidation, to reduce peptides toxicity and enhance their inhibitory effect.

208 - Prevalence and Molecular Profiling of Merkel Cell Polyomavirus in patients with Monkeypox virus infection

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Prevalence and Molecular Profiling of Merkel Cell Polyomavirus in Patients with Monkeypox Virus Infection

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Introduction: Recent Monkeypox Virus (MPXV) outbreaks have raised concerns regarding co-infections with other viral agents and their potential clinical implications. Merkel Cell Polyomavirus (MCPyV), a ubiquitous virus, is identified as the causative agent of Merkel Cell Carcinoma, an aggressive form of skin cancer with poor outcomes, particularly in immunosuppressed patients. Here, we investigated the positivity rate, viral loads, and molecular state of MCPyV among men who had sex with men (MSM) testing positive for MPXV infection.

Materials and Methods: Oropharyngeal and anal swabs were collected during the acute phase of MPXV infection from 66 individuals (median age: 42 y, IQR=33-47). MCPyV detection and viral load quantification were performed by qPCR. MCPyV-positive samples were further analyzed to assess viral integration and large T antigen (LT) truncation. Again, RT-PCR was used to assess the expression of LTAg and VP1 transcripts, and RT-qPCR was employed to detect MCPyV-encoded microRNAs.

Results: MCPyV DNA was detected in 23/66 (34.8%) MPXV-positive patients. MCPyV DNA was found in 10/41 (24.4%) oropharyngeal and 14/44 (31.8%) anal swabs with a mean viral load of $1.3x10^2$ and $2.1x10^3$ copies/mL, respectively. Of MCPyV-positive oropharyngeal samples during

the acute MPXV phase, 4/10 (40%) remained positive at follow-up (mean viral load of $1.16x10^3$ copies/mL). Among anal samples, MCPyV was detected in 9/32 (28.1%) non-lesional and 5/12 (41.6%) lesional swabs (mean viral load of $2.6x10^3$ and $1.15x10^3$ copies/mL, respectively). No viral integration was observed, and a full-length LT was amplified in all specimens. LTAg and VP1 transcripts were expressed in MCPyV-positive samples. Expression of mcv-miR-5p was found in 3/10 (30%) oropharyngeal and 5/14 (35.7%) anal MCPyV-positive swabs collected during the acute phase. At follow-up, mcv-miR-5p was detected in 3/4 (75%) MCPyV DNA-positive oropharyngeal samples. Among MCPyV-positive individuals, 12/31 were also HIV-positive; other co-infections included Syphilis (1/5), Mycoplasma (4/5), Ureaplasma (1/4), and anal HPV (2/5).

Discussion and Conclusions: This is the first report describing MCPyV prevalence among MPXV-positive individuals. The detection of MCPyV DNA and the expression of both early and late viral transcripts supports the hypothesis of active viral replication in both the examined anatomical districts during the acute phase of MPXV infection. Notably, the high recovery of MCPyV in anal swabs from MSM may support the plausible sexual transmission of the virus. Moreover, the detection of MCPyV DNA and miRNAs in the acute phase and partially in the follow-up may suggest a transient reactivation of the virus during MPXV infection followed by the establishment of a persistent/latent state.

213 - DENG-VAC study: preliminary data on immunogenity of Qdenga vaccine versus natural infection after 12 months post-vaccination.

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DENG-VAC study: preliminary data on immunogenity of Qdenga vaccine versus natural infection after 12 months post-vaccination.

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Introduction Dengue is a mosquito-borne disease caused by the dengue virus (DENV), whose global incidence has grown dramatically in recent decades. The aim of the study is to monitor the immunogenicity of the QDENGA vaccine compared to acute DENV natural infection.

Materials and Methods This is a single-center prospective observational study of a cohort of vaccinees with The QDENGA vaccine that started in March 2024. The study population was stratified as follows: a) DENV seronegative vaccinees (naïve); b) DENV seropositive vaccinees (experienced); c) unvaccinated subjects enrolled during acute DENV infection. Blood samples were collected at T0, T3, and T12 after the first vaccine dose or infection. A micro-neutralization test based on the DENV-2 isolate was performed to measure neutralizing antibodies (nAb). Anti-DENV IgM were evaluated by ELISA test. Monocytes (M) and both myeloid and plasmacytoid dendritic cells (mDC and pDC, respectively) were analyzed by multiparametric flow-cytometry. Data were analyzed by Kruskal- Wallis (Dunn's), Mann-Whitney, and Wilcoxon tests, as appropriate. A $p<0.05$ was considered significant.

Result A total of 140 subjects were enrolled, no serious adverse reactions were observed. The first dose of the vaccine induced anti-DENV nAb in 39/42 (92.8%) individuals from T0 to T3 ($p<0.0001$), specifically in 23/23 (100%) and 16/19 (84.2%) naïve and experienced subjects, respectively. nAb increased from T0 to T3 both in naïve (22.3-fold, $p<0.0001$) and experienced (8-fold, $p<0.067$); however, higher levels of anti-DENV nAb were observed in experienced vs

naive subjects at T0 ($p<0.0001$) and T3 ($p<0.0001$). At T3, the experienced subjects showed higher nAb titers as compared to the naïve ($p=0.0007$) and to DENV infected ($p=0.0078$) patients. At T12, all vaccinated subjects showed positive nAb. Finally, at T3 naïve showed higher levels of anti-DENV IgM as compared to the experienced ($p=0.0006$) and DENV infected patients ($p=0.0235$).
M and mDC cell frequencies were similar in all groups before vaccination. At T3, a significant increase of M was reported ($p=0.02$) in experienced subjects. In DENV patients, mDC decreased compared with other groups ($p=0.0005$), while pDC showed an increase respect to naïve and experienced individuals ($p<0.001$).
Discussion and Conclusion Our preliminary data showed that the QDENGA vaccine is safe and immunogenic. The first vaccine dose elicits a functional antibody response in naïve and experienced subjects. One year after QDENGA vaccination, this response is detectable in all vaccinated subjects. An increase of M is observed in experienced subjects following the first vaccine dose, while an increase of pDC only in DENV patients is reported. Further analyses on the current cohort are ongoing.

221 - Antiviral and anti-oxidant activity of Lavandula angustifolia extract-loaded vesicles for the treatment of HSV-1 infections

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Antiviral and anti-oxidant activity of Lavandula angustifolia extract-loaded vesicles for the treatment of HSV-1 infections

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Introduction

Herpes simplex virus type 1 (HSV-1) is a widespread pathogen responsible for recurrent mucocutaneous infections. *Lavandula angustifolia* Miller (*L. angustifolia*) is known for its potent anti-inflammatory, antioxidant, wound-healing and antiviral properties. However, its low stability under certain conditions limits its therapeutic potential. We evaluated the potential of a biotechnologically derived *L. angustifolia* extract, incorporated into vesicular carriers for topical application.

Materials and Methods

Four phospholipid-based nanocarriers namely liposomes, glycerosomes, hyalurosomes, and glycerohyalurosomes, were prepared with the extract. Vesicles were characterized for size, zeta potential, entrapment efficiency and long-term stability. Biological studies on Normal Human Dermal Fibroblasts (NHDFs) and Vero cells evaluated biocompatibility, protection from oxidative stress and antiviral activity against HSV-1 via cytopathic effect assays.

Results

Formulations ranged from 58 to 80 nm in diameter, with high L. angustifolia extract entrapment (≥94%, except glycerohyalurosomes: 54%) and strongly negative zeta potential all maintaining a highly negative surface charge, ensuring stability and reduced aggregation. Glycerosomes and glycerohyalurosomes maintained stability for 12 months. All formulations were non-cytotoxic and improved NHDFs viability under oxidative stress. In antiviral tests, they showed strong protection (up to 100%) during and post-infection treatment.

Discussion and Conclusions

The vesicles exhibited dual anti-oxidant and antiviral action, especially glycerol- and hyaluronate-based systems. These promising results support their potential as topical treatments for HSV-1 lesions by enhancing bioactives availability and stability. In vitro studies on NHDFs demonstrated that all formulations were biocompatible and enhanced cell viability under oxidative stress. Furthermore, these formulations showed promising antiviral activity against HSV-1, particularly when applied during or post-infection. Overall, these phospholipid vesicles offer a dual therapeutic approach, combining antioxidant, and antiviral effects, positioning them as promising candidates for the treatment of HSV-induced skin lesions and related conditions.

223 - Neutralising and Binding Antibodies in Mpox Patients and MVA-BN Vaccine Recipients

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Neutralising and Binding Antibodies in Mpox Patients and MVA-BN Vaccine Recipients

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Introduction

Monkeypox virus (MPXV), a zoonotic Orthopoxvirus (OPXV), causing mpox disease, re-emerged globally in 2022, prompting widespread public health interventions as the deployment of third-generation smallpox vaccines (e.g. JYNNEOS) in at risk population. Given the genetic relatedness of MPXV to Variola, Vaccinia (VACV), and Cowpox (CPXV) viruses, serological cross-reactivity complicates differential diagnosis and epidemiological surveillance.

Our study aimed to assess OPXV-specific IgA, IgM, and IgG responses and neutralising antibodies (NAbs) against MPXV, VACV and CPXV in mpox patients and MVA-BN-vaccinated subjects.

Materials and Methods

Cohort comprised mpox cases (n=15) and HCWs vaccinated with JYNNEOS (n=6), including previously vaccinated for smallpox who received only one dose of MVA-BN (n=2). Longitudinal sampling was conducted at early (T1), intermediate (T2), and late (T3) phases post-infection and until 2 years post-vaccination (PV). Plaque reduction neutralisation test (PRNT) against MPXV, VACV, and CPXV was performed alongside a fluorescent reduction neutralization test (FRNT) by using MVA-eGFP recombinant virus to evaluate NAb levels. OPXV-specific IgA, IgM, and IgG titers were evaluated through a home-made indirect immunofluorescence assay (IFA).

Results

$PRNT_{50}$ titers were similar across MPXV, VACV, and CPXV, complicating the NAbs differential detection between these viruses. In some cases, VACV titers were higher than MPXV. No significant increase in NAbs was observed in previously smallpox-vaccinated mpox patients

compared to OPXV-naïve individuals, suggesting that prior immunity does not substantially alter the immune response during mpox infection.
IgA titers peaked at T1, preceding IgM, while both IgA and IgM antibodies declined at T3. In contrast, IgG levels steadily increased over time. These findings suggest IgA as early serological marker, potentially offering diagnostic utility in the initial stages of infection.
In JYNNEOS vaccinated subjects, NAbs against VACV and MVA-BN were significantly higher than those against MPXV or CPXV. This allowed clear differentiation between vaccinated individuals and those with natural infection based on differential PRNT and FRNT titers. IgG response was robust shortly after vaccination and remained elevated even 2 years PV.

Discussion and Conclusion

Despite high OPXV cross-reactivity, differential PRNT/FRNT titers and specific antibody kinetics, particularly early IgA responses, support refined serodiagnostic strategies. MVA-BN vaccination elicits robust cross-NAbs without inducing IgM/IgA in asymptomatic recipients, aiding in exposure differentiation. The evaluation of differential neutralizing titers may serve as a valuable diagnostic tool for retrospective analysis of OPXV outbreaks.

224 - Ongoing B3 lineage circulation in and around Milan (northern Italy) in early 2025: is the canary in the coal mine dying again?

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Ongoing B3 lineage circulation in and around Milan (northern Italy) in early 2025: is the canary in the coal mine dying again?

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Introduction. After documenting an epidemic activity of measles in 2024, sustained by the multiple introductions of different lineage of genotype D8, as Subnational Reference Laboratory of the Italian measles and rubella surveillance network (MoRoNet), here we report a new surge of measles in the area under surveillance (Milan and surrounding areas), now driven by a single lineage of genotype B3 causing indigenous cases.

Materials and Methods. Laboratory confirmation of suspected measles cases (fever and rash) was performed through serological testing for measles-specific IgM antibodies in acute-phase serum samples (collected within 1–10 days of rash onset), and/or through the detection of measles virus (MeV) RNA in urine samples and/or oropharyngeal swabs. MeV genotyping was performed by sequencing the N-450 genomic region.

Results. Between January and April 2025, 28 measles genotype B3 cases were identified over 33 laboratory-confirmed, occurred mainly among young unvaccinated adults (23/33, 70%).

Cases were mostly indigenous (23/28, 82.1%). Imported cases (5/28, 17.9%) were travellers returning from Morocco, France, and Spain. All the genotype B3 strains belonged to the same lineage (within-clade identity: 99.3–100%), and clustered with strains circulating in 2022-2025 in Morocco, Iran, and the Netherlands. Starting from week 05-2025, the B3 lineage, presumably introduced by a traveller returning from Morocco, spread in Como, Milan, and Monza-Brianza provinces, causing 11 sporadic and 16 outbreak-related cases. Two cases occurred in healthcare settings, and 3 in a nursery school. Four individuals had recently attended mass gathering events, supporting potential silent transmission chains.

Discussion and Conclusion. The circulation of a single genotype B3-lineage, with nearly identical sequences and limited links to imported cases, reflects a transmission pattern compatible with reintroduction and possible progression toward endemicity. Although a continuous chain of transmission could not be fully reconstructed, the genetic profile and temporal and spatial distribution of cases suggest ongoing circulation over 3 months.
In line with national data, the ongoing outbreak is mainly affecting unvaccinated adults, suggesting that the high vaccination coverage in Lombardy —96% and 94% for 1 and 2 doses, respectively— has successfully limited transmission in younger age groups.
Our findings highlight the risk of re-establishing endemic transmission, i.e. uninterrupted circulation for over 12 months. Although this threshold has not yet been reached, the current scenario underscores the urgency of reinforcing immunisation strategies, closing immunity gaps, and enhancing molecular surveillance to promptly detect and respond to transmission events.

235 - Environmental Surveillance revealed emergence of recombinant Echovirus 11 in Sicily

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– **Topic**: Patogeni emergenti e riemergenti

– **Title**: **Environmental Surveillance revealed emergence of recombinant Echovirus 11 in Sicily**

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Introduction

A novel strain of Echovirus 11 (E11) lineage 1, initially emerged in France in July 2022, has spread to several European countries, becoming epidemiologically relevant. Wastewater-based surveillance (WBS) has proven useful for epidemiological monitoring, allowing for the tracking of viral spread. In Sicily, WBS has detected the emergence and sustained circulation of this new E11 strain since October 2022. Although enterovirus infections are typically asymptomatic and self-limiting, recombinant events have been linked to increased disease severity, chiefly in infants. This study aimed to perform whole-genome analysis on E11 strains identified through environmental surveillance in Sicily during 2022–2023.

Materials and Methods

A total of 266 wastewater samples were collected from wastewater treatment plants (WWTPs) in Palermo, Trapani, Messina, Syracuse, and Avola, and one Pre-Removal Detention Centre for migrants in Trapani. Wastewater sampling and virus concentration were performed following WHO guidelines. All NPEs were confirmed by amplification of the 5'-UTR region and subsequently genotyped by sequence analyses of the VP1 segment of the genome. To investigate the genetic evolution of circulating E11 lineage 1 strains, whole-genome sequences of eight representative Italian isolates were compared with complete E11 genome sequences available in GenBank (=91).

Results

NPEs were detected in 17.6% of the 266 wastewater samples analysed. E11 was the most prevalent virus (76.6%) across all monitored sites. The novel E11 lineage 1 strain accounted for 72.2% of the NPEs, with increased detection over time (from 42.8% in 2022 to 79.3% in 2023). Phylogenetic analysis of the VP1 gene revealed a heterogeneous population of E11 strains, clustering in D5 genogroup. On whole-genome sequencing (P1, P2A-2C and P3A-B, 3C and 3D regions) of eight selected Italian strains, multiple recombination events were mapped, affecting either the P2 or P3 regions. Recombination involved E11 strains of different lineages and non-E11 enteroviruses, including E80, E85, and Coxsackie B4 and B5.

Discussion and Conclusions

These results highlight the critical role of whole-genome sequencing in understanding the genetic evolution of emerging enteroviruses. Moreover, they underscore the effectiveness of WBS as a timely and non-invasive approach for detecting and monitoring the emergence or re-emergence of new variants with potential public health implications.

254 - Environmental Genomic Surveillance reveals emerging circulation of Echovirus 6 in Sicily during 2024

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Environmental Genomic Surveillance reveals emerging circulation of Echovirus 6 in Sicily during 2024

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Introduction: In recent years, Echovirus type 6 (E6), member of the Enterovirus B species, has shown a marked increase in infection rates across Europe, raising public health concerns. This increase was particularly associated with neurological symptoms, highlighting the virus's neurotrophic potential. While its epidemiological role remains unclear, wastewater-based surveillance has proven effective for early virus detection. Since 1996, the Sub National Reference Laboratory (SNRL) at PROMISE University Department in Palermo participates to the global Polio (PV) eradication surveillance network, coordinated by the Italian Ministry of Health and National Institute of Health. During the environmental surveillance for PV during 2024, an increase of E6 detection was observed, in Sicily. In this study, E6 isolates were genetically characterized through the analysis of the VP1 genomic region to investigate the role of genetic evolution in the emergence of these viruses.

Material and Methods: A total of 150 wastewater samples were collected in 2024 twice a month (15 days apart) for entire surveillance period. The wastewater samples were subjected to clarification and concentration with the dextran/PEG biphasic separation method, followed by inoculation into RD and L20B cell lines to detect enteroviruses (EVs), in accordance with WHO-recommended protocols for PV surveillance. All isolates of non-polio enterovirus (NPEV), recovered on RD cells, were confirmed by amplification of the 5'UTR gene region and genotyped by sequence analysis of the VP1 gene.

Results: During the study period, the 18.7% of the wastewater samples were positive for NPEV and E6 represented the predominant strain (66.7%). The phylogenetic analysis of the VP1 sequences showed that 86.6% of the Sicilian E6s clustered together with the strains detected in France in 2022, showing 96.5 and 100% of nt id to each other and 96.1 -98.4% respect to French strains, respectively. One E6 strain detected in Messina clustered with strains detected

in Florida in 2024 (97.3 % of nt id) and a strain from Syracuse clustered in a separate branch showing more than 96.4 of nt id respect to all other strains available in GenBank. Interestingly, none of the Sicilian sequences resembled the E6 clusters described in Europe until 2016.

Discussion and Conclusions: The increasing incidence of E6 infections, often associated with severe clinical outcomes, highlights the importance of continuous surveillance with further epidemiological and genetic studies. In this context, wastewater-based surveillance is confirmed to be a sensitive tool for the early monitoring of emerging enteroviruses.

256 - Investigating The Therapeutic Potential Of Two Microbial Derivatives: The Antiviral Activity Of Brefeldin A And Monensin Against Toscana Virus.

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Investigating The Therapeutic Potential Of Two Microbial Derivatives: The Antiviral Activity Of Brefeldin A And Monensin Against Toscana Virus.

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Introduction. Toscana virus (TOSV) is an arthropod-borne virus belonging to the *Phlebovirus* genus within the *Phenuiviridae* family. It is transmitted by blood-feeding phlebotomine sandflies in countries surrounding the Mediterranean Sea and is increasingly being detected beyond this region. In humans, TOSV infection typically results in a mild febrile illness, although cases of meningitis and encephalitis have also been reported. Currently, there is no specific treatment or human vaccine available. Thus, this study aims to investigate the antiviral activity of two microbial derivates, Brefeldin A (BFA) and Monensin (MN), against TOSV. Brefeldin A is a fungal metabolite that disrupts intracellular membrane trafficking at the ER-Golgi interface. Monensin, a metabolite produced by *Streptomyces cinnamonensis*, acts as a Na^+/K^+ ionophore and impairs trans-Golgi function by inhibiting protein transport from the medial to the trans-cisternae of the Golgi apparatus. **Materials and Methods**. To exclude the possibility that BFA and MN exert cytotoxic effects, various concentrations of both compounds were evaluated using the 3-[4,5-dimethylthiazol-2-yl]-2,5 2,5-diphenyl tetrazolium bromide (MTT)

assay. A plaque reduction assay was then conducted: cell monolayers were infected with TOSV at a multiplicity of infection (MOI) of 0.01. Following virus adsorption at 37°C for 1 hour, the inoculum was removed, and BFA or MN were added and incubated for different time points: 1, 4, 6, 12, and 24 hours. **Results.** Our data suggest that MN effectively inhibits TOSV infection at early time points. Specifically, we observe a complete inhibition of the infection at 4 hours post-infection (hpi) in the range of non-cytotoxic concentrations from 6.25 to 25 µM. In contrast, BFA reduces TOSV infection at later time points at concentrations in the nanomolar range. **Discussion and Conclusion**. In conclusion, we identified two promising natural compounds, BFA and MN, that show antiviral activity affecting TOSV intracellular infection. Further studies will be required for a better understanding of their precise mechanism of action.

268 - Echovirus 11 infection in vitro: dissecting inflammatory pathways in hepatic and CNS-derived cell lines

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Echovirus 11 infection in vitro: dissecting inflammatory pathways in hepatic and CNS-derived cell lines

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Introduction: Echovirus 11 (Echo11) is a single-stranded RNA enterovirus (EV) of the *Picornaviridae* family, associated with significant neonatal morbidity and mortality. It is transmitted vertically (in utero or at delivery) or acquired postnatally through nosocomial spread. Clinical manifestations range from asymptomatic infections to severe, life-threatening diseases. In neonates, Echo11 infection can lead to sepsis, myocarditis, meningitis, and most notably, fulminant hepatitis characterized by jaundice, hepatomegaly, coagulopathy, metabolic acidosis, and multiorgan dysfunction. Since 2022, 19 severe neonatal Echo11 cases, including 9 deaths, have been reported across several EU/EEA countries. The UK and Japan have also reported fatal cases. Molecular analyses identified a common viral lineage in cases of neonatal acute liver failure (ALF) across Europe and Japan between 2022 and 2024.
Material and Methods: To assess eventual effects on brain cells, a 2D murine blood–brain barrier (BBB) model was established in combination with a neuron/astrocyte/microglia tri-culture. Cells were infected with Echo11 (ATCC VR-41, Gregory strain) at a multiplicity of infection (MOI) of 0.05. BBB integrity was evaluated 24 hours post-infection using transendothelial electrical resistance (TEER) measurements, FITC-dextran permeability assays, and ZO-1 immunofluorescence. For hepatic analysis, HepG2 cells were infected with Echo11 at the same MOI. Viral replication was assessed by $TCID_{50}$, RT-PCR, and flow cytometry. Protein expression was evaluated by Western blotting, and cytokine expression was analyzed by RT-PCR and flow cytometry.
Results: Our results show that Echo11 infection induces a marked cytopathic effect in BBB endothelial cells, as evidenced by a significant reduction in TEER values. Confocal imaging of ZO-1 staining further confirmed disruption of tight junctions, indicating compromised BBB integrity.
A similar cytopathic effect was observed in Echo11-infected HepG2 cells. In both BBB and hepatic cell models, Echo11 infection modulated the JAK/STAT signaling pathway, triggered robust cytokine production and altered the expression of NF-κB and Nrf2, key regulators of inflammatory and oxidative stress responses.
Discussion and Conclusions: This study highlights the ability of Echo11 to disrupt BBB integrity and cause cytopathic effects in hepatic and endothelial cells. Activation of the JAK/STAT pathway and strong cytokine responses suggest that inflammation plays a key role in virus-induced tissue damage. These findings may help to explain the severe outcomes seen in neonatal infections and support the need for targeted therapeutic approaches.

285 - Molecular Detection and Clinical Correlation of Trichomonas tenax and Entamoeba gingivalis in Periodontal Disease

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Molecular Detection and Clinical Correlation of *Trichomonas tenax* and *Entamoeba gingivalis* in Periodontal Disease

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Introduction

Periodontal disease is a prevalent chronic inflammatory condition traditionally attributed to bacterial dysbiosis. However, recent research has highlighted the potential role of eukaryotic microorganisms as emerging contributors to oral pathogenesis. Among them, *Trichomonas tenax* and *Entamoeba gingivalis,* long considered oral commensals, are increasingly detected in diseased periodontal sites, raising questions about their involvement in disease onset and progression.

No data have been available so far regarding the presence and clinical relevance of these protozoa in the Italian population. This study aimed to evaluate the prevalence of these protozoa in individuals with different periodontal conditions and assess their correlation with clinical periodontal parameters.

Materials and Methods

A prospective observational study was conducted on on 51 healthy subjects and 105 patients with gengivitis between July 2024 and January 2025. Subgingival plaque samples were collected from periodontal pockets and analyzed using direct microscopy, culture, and PCR-based molecular methods to detect *T. tenax* and *E. gingivalis*. Clinical periodontal parameters—including probing depth (PD), bleeding on probing (BoP), plaque index, and clinical attachment loss (CAL)—were recorded. The association between the presence of protozoa and major risk factors, namely smoking and diabetes, was also assessed.

Results

T. tenax was identified in 9.6% and *E. gingivalis* in 21.7% of the total sample. Coinfection was detected in 5.1% of cases. Among healthy individuals, 6 out of 51 (11.8%) tested positive for at least one protozoan, whereas in the patient group, 35 out of 105 (33.3%) were positive ($p = 0.0037$). *T. tenax* and *E.gingivalis* were significantly associated with sites presenting deeper PD, elevated BoP, and abundant plaque.

Discussion and Conclusions

The notable prevalence of oral protozoa in patients with periodontal disease has been described and suggests a potential association with clinical indicators of disease severity. Our findings support the hypothesis that *T. tenax* and *E. gingivalis* may act as emerging oral pathogens contributing to the progression of periodontal disease. Their ability to persist in inflamed sites, interact with host immunity, and coexist with pathogenic bacteria highlights their possible role as pathobionts within a polymicrobial biofilm. These results underscore the need for further research into their pathogenic mechanisms and advocate for including protozoa in the broader microbial surveillance of periodontal disease.

293 - H1N1 influenza virus activates neurodegenerative pathways in brain cells

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H1N1 influenza virus activates neurodegenerative pathways in brain cells

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Introduction: H1N1 influenza A virus (IAV) causes both seasonal epidemics and worldwide severe pandemics, productively infecting the epithelial cells of the upper respiratory tract, and eventually the lower respiratory epithelia, leading to severe complications. IAV occasionally affects the central nervous system, causing acute neurological manifestations, mainly mediated by neuroinflammation, and possible long-term sequelae that have been related to neurodegenerative diseases. Recent findings from *in vivo* studies in a transgenic mouse model of Alzheimer's disease (AD) suggest that IAV infection may accelerate the disease progression. On the contrary, some papers report that amyloid-beta peptides (Aβ), key triggers for AD pathogenesis, can efficiently inhibit IAV replication in the brain. However, the effects of IAV infection on the brain both in the short- and long-term remain elusive.
Materials and Methods: Primary murine neuron-glia co-cultures, human neuroblastoma SH-SY5Y cells, and murine organotypic brain cultures were infected with different doses of IAV or mock solution. Cells and tissues were analyzed at various times post infection to evaluate the expression levels of proteins related to neurodegeneration, i.e., Amyloid Precursor Protein

(APP), Aβ, tau protein and its phosphorylated forms (ptau), the post-synaptic markers PSD-95, interleukin-1β (IL-1β) and autophagic components by western blot, confocal immunofluorescence, and virological assays.

Results: We found that IAV infection induced APP downregulation and accumulation of Aβ in both primary brain cells and SH-SY5Y cells. These events were partially rescued in those cells undergoing infection in the presence of a β-secretase inhibitor, thus suggesting that the virus may promote APP amyloidogenic processing. In parallel, we found that IAV modulates some autophagic components, likely contributing to Aβ accumulation. We also detected an increase in intracellular IL-1β and a significant decrease in PSD95 following IAV infection, indicating that the virus induces neuroinflammation and synaptic damage within the acute infection. On the contrary, we did not find changes in tau phosphorylation, but ptau was mainly found decentered toward the nucleus of infected neurons, suggesting that it may play a neurotoxic role in such location. Finally, we set up an *ex vivo* model of IAV infection in murine organotypic brain cultures, herein inducing the accumulation of Aβ, the increase of IL-1β, and the downregulation of PSD-95.

Conclusions: Overall, our results indicate that IAV infection activates neurodegenerative pathways within the acute infection. Further studies are required to unravel the role of cell response in such events and their long-term potential.

303 - Direct and indirect contribution of ORF7b and ORF8 in dysregulation of NF-κB and IFN pathways.

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Direct and indirect contribution of ORF7b and ORF8 in dysregulation of NF-κB and IFN pathways.

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Aims and hypothesis: The aim of this study is to investigate the effect of a deletion of 426 nucleotides (nt) in the ORF7b-ORF8 gene region of SARS-CoV-2 and the possible modulation of IFN-β and NF-κB pathways. This deletion was found in SARS-CoV-2 infected patients and healthcare personnel of the AOUI Verona hospital. We hypothesize that this deletion could alter IFN-β and NF-κB pathways both directly, through protein-protein interactions, and indirectly through the alteration of host alternative splicing.

Scientific rational: ORF7b viral protein could interact with cellular MAVS protein, and it is suggested that it could be involved in the deregulation of IFN-β pathway induction. Since IFN-β and NF-κB pathways cross-talk, we could hypothesize that the deletion of 426 nt that leaves the truncate form of ORF7b and the last 3 amminoacids of ORF8 viral proteins could deeply impact on the deregulation of these two pathways, limiting the efficacy of the host antiviral response.

Preliminary data: We identified the 426 deletion in the SARS-CoV-2 genome in 6 infected patients. All of them showed a milder phenotype compared to classical COVID-19 symptoms, as previously reported. This feature and the longer positivity period are compatible with a decrease of immune response due to alteration of IFN-β and NF-κB pathways. We cloned

ORF7b and ORF8 wild type genes and ORF7b-ORF8 with the deletion in a pGEM®-T Vector (Promega). These constructs are the basis for the next planned functional assays.

Future perspectives: We will perform transactivation assays in Calu3 cell line to analyze the modulation of IFN-β and NF-κB pathways by measuring their promoter transactivation in dual luciferase assays and normalizing our data with Renilla luciferase internal control. We will proceed to confirm our results by analyzing IFN-b and NF-κB transcript expressions. In addition, we will compare the interaction of ORF7b-ORF8 wt and mutated proteins with the component of IFN-β and NF- κB cascades by co-immunoprecipitation experiments and confirm with localization studies. The study will be completed by an NGS sequencing to analyze the expression levels of alternative transcripts in the presence of ORF7b and ORF8 wild type or their truncated forms and to assess the real role of these two viral proteins in altered expression of cell transcripts as reported for ORF8. This approach will allow us to elucidate the mechanism by which SARS-CoV-2 could promote viral persistence and induce a milder immune response through modification of ORF7b and absence of ORF8.

307 - Exploration of activity on Multi-Drug Resistant microorganisms of a panel of different molecules from an IT discovery campaign

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Exploration of activity on Multi-Drug Resistant microorganisms of a panel of different molecules from an IT discovery campaign

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Aim of the Study and Hypothesis

The central focus of this research is to evaluate the antibacterial potential of a pool of 37 low-molecular-weight chemical compounds selected considering their chemical and physical properties against three clinically significant multidrug-resistant (MDR) Gram-negative bacterial strains: *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The hypothesis of this study is that, within this set of molecules, there are candidates that may exert significant, even broad-spectrum inhibitory activity by virtue of their ability to overcome the physiological barriers of these microorganisms and interact with crucial bacterial targets.

Scientific rationale

According to the WHO, MDR Gram-negative bacteria represent a major threat to human health. Their resistance is aided by external selective membranes, efflux pumps and the pathogen's ability to produce inactivating enzymes. In this context, there is an urgent need for new molecules with innovative mechanisms of action. IT discovery campaigns based on libraries of

chemically diverse compounds offer a promising alternative since these molecules are potentially able to cross the bacterial membrane and hit vital targets for bacterial survival.

Preliminary data

37 structurally diverse, drug-like molecules characterised by a molecular weight ranging from 200 to 500 g/mol and a lipophilic index between 1 and 3, were tested. By broth microdilution assays, six compounds exhibited inhibiting action towards all three bacterial strains, with minimum inhibitory concentration values between 15,62 and 500 µg/ml. Twelve compounds were found to be active on two strains, eleven on only one, while eight displayed no activity. *Pseudomonas aeruginosa* proved to be the most susceptible strain to these molecules (27 active compounds), followed by *Acinetobacter baumannii* (15) and *Klebsiella pneumoniae* (10). Results underline a variability associated with different membrane permeabilities and mechanisms of resistance.

Future perspective

The preliminary results, although limited, pave the way for numerous future developments, thus overcoming the limitations associated with traditional approaches, often based on modifications of already known scaffolds. It will be necessary to identify the mechanisms of action of the most promising compounds and perform molecular target binding assays. Potential synergism with pre-existing antibiotics will be tested to expand the already available therapeutic repertoire and reduce the likelihood of the emergence of new resistance. This study provides a basis for the development of new antimicrobial strategies, contributing to the identification of molecules with clinical potential in contrasting infections by multidrug-resistant Gram-negative bacteria.

308 - Measles in the Emilia-Romagna Region: the crucial role of virological diagnosis and molecular characterization of circulating viral strains

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Measles in the Emilia-Romagna Region: the crucial role of virological diagnosis and molecular characterization of circulating viral strains

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Introduction. Since early 2024, a significant increase in measles cases has been reported in the European Region, including Italy. This study presents data from surveillance activities by the Emilia-Romagna Region (ERR) Reference Laboratory, which is part of MoRoNet, the national network of reference laboratories for measles and rubella.
Materials and Methods. From January 2024 to date, 739 samples (247 urine, 246 oral fluids, and 246 sera) from 257 suspected measles cases were tested. Molecular diagnosis was performed using a *homemade* multiplex real-time PCR for the simultaneous detection of measles virus (MV) and rubella virus (RV) RNA in urine and oral fluid. Serological test was performed using the LIAISON® Measles IgG/IgM kit (DiaSorin). Molecular characterization of MV

strains was carried out by analyzing the highly variable 450-nucleotide region of the N gene through Sanger sequencing.

Results. Among 257 suspected cases, 166 (64.6%) were confirmed using at least one of the two diagnostic methods. The remaining 91 cases (35.4%) were classified as non-cases based on negative MV and RV results. Median age of confirmed cases was 34 years (range: 8 months - 67 years). Vaccination status was known for 157 (94.6%) of the cases: 143 (91.1%) were unvaccinated, 8 (5.1%) had received one dose, and 6 (3.8%) had received two doses. Most confirmed cases (59.6%) were from an ERR area with several vaccine-hesitant communities. MV strains characterization was performed in 157 out of 158 (99.4%) confirmed cases, revealing that 83.4% of strains belonged to genotype D8 and 16.6% to genotype B3. Phylogenetic analysis identified 8 distinct variants belonging to D8 genotype and 4 belonging to B3 genotype. Endemic circulation was observed in 87.2% of cases, while 12.8% of strains were associated with imported cases. Clinical data were available for 56 of the 166 confirmed cases (33.7%), of whom 17 (30.3%) developed complications such as pneumonia, pneumonia associated with respiratory failure, otitis, and diarrhea. One pediatric patient developed pericarditis. In addition, due to the recent resurgence of parvovirus B19 infection in Europe, 67 of the 91 non-cases were tested for parvovirus B19 DNA, with 27 (40.3%) resulting positive.

Discussion and Conclusions. Our results highlight: *i)* the crucial role of virological diagnosis and active surveillance in identifying measles cases and distinguishing endemic from imported cases; *ii)* the widespread circulation of MV in a geographic area with vaccine-hesitant communities; *iii)* the high frequency of complications associated with MV infection in susceptible individuals; *iv)* the importance of performing a differential diagnosis with other pathogens responsible for febrile-rash diseases.

315 - Newly discovered antimicrobial peptides with antiviral potential against Bunyaviridae family

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Newly discovered antimicrobial peptides with antiviral potential against *Bunyaviridae* family

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Introduction:Toscana virus (TOSV) and Schmallenberg virus (SBV) are arthropod-borne viruses from the *Bunyaviridae* family, posing significant threats to human and animal health. TOSV, endemic to the Mediterranean region, is a notable human pathogen also detected in various animals, suggesting potential zoonotic reservoirs. SBV emerged in Europe in 2011, affecting ruminants and causing reproductive issues, with substantial economic implications. Host defense peptides (HDPs), particularly those derived from scorpion venom, are gaining attention for their antiviral potential.

In the present study, we investigated the antiviral activity of two scorpion-derived peptides, pantinin-1 and pantinin-2, against TOSV and SBV.

Materials and methods: The peptides were prepared using solid-phase peptide chemical synthesis, and evaluated for their cytotoxicity on human neuronal cell line U87-MG, Crandell-Rees Feline Kidney (CRFK**),** canine fibroblast cell line (A72), baby hamster kidney cells (BHK-21) using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. After selecting the non-toxic concentrations of both peptides, the antiviral activity against TOSV and SBV was evaluated by plaque reduction assay and observation of cytopathic effect. The results were further validated by real-time PCR, analysing the M segment coding for viral glycoproteins.

Results: Both peptides exhibited a potent virucidal effect against TOSV and SBV, with half-maximal inhibitory concentration (IC50) values of 12 μM for pantinin-1 and 6 μM for pantinin-2. In addition to their virucidal activity, we also examined the ability of pantinin-1 and pantinin-2 to block TOSV infection in human cells. Using U87-MG cells, we demonstrated that both peptides could effectively interfere with the infection process. This is particularly important, as TOSV has been associated with severe neurological diseases, including encephalitis and meningoencephalitis, which primarily affect the brain. The molecular data confirmed antiviral action, showing a complete reduction at higher tested concentrations (50 and 25 μM) in gene expression.

Discussion and conclusion: Given the increasing prevalence of emerging viruses and the growing concern over antibiotic resistance, peptides like pantinin-1 and pantinin-2 offer a novel approach to combating viral infections, particularly in cases where traditional antiviral therapies are limited or ineffective. In conclusion, our study identifies pantinin-1 and pantinin-2 as potential antiviral candidates for preventing or treating TOSV and SBV infections. Future research is needed to further explore the mechanisms of action of these peptides, their efficacy in vivo, and their potential for development into therapeutic agents.

320 - Three years of surveillance of human dengue virus infections in Lombardy, 2023-2025

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Three years of surveillance of human Dengue virus infections in Lombardy, 2023-2025

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Introduction: In recent decades, rising temperatures, tropical rainfall, and resistance to insecticides are the main factors that have facilitated the geographic expansion of vectors, and in particular mosquitoes of the genus Aedes. This has contributed to a continuous and progressive increase in Dengue virus (DENV) cases in Italy. The Microbiology and Virology department, IRCCS Fondation Policlinico San Matteo of Pavia, as the Regional Reference

Center for the diagnosis of arboviruses is actively involved in the human surveillance of DENV cases. The main aims of this study were: i) to conduct epidemiological surveillance of DENV cases from 2023 to 2025, ii) phylogenetic and molecular characterization of autochthonous DENV cases detected in the Lombardy region.

Materials and methods: In the three-year surveillance period, January 2023 to date, 143 cases of DENV have been diagnosed. Molecular diagnosis was made by viral RNA detection using virus-specific Real Time RT-PCR and a Hemi-nested RT-PCR *Flavivirus*. Serological diagnosis in order to assess the presence of specific antibodies of the IgM and IgG classes against DENV was performed by DENGUE VIRCLIA® IgM/IgG MONOTEST (Vircell, Granada, Spain) on serum samples. Complete genomes of autochthonous DENV cases were obtained by metagenomic sequencing (mNGS).

Results: In the three year surveillance period, 583 suspected cases of DENV were investigated and 143 (143/583, 24%) were confirmed. In 2023, 77 cases (77/220, 35%) were diagnosed, 37 (37/77, 48%) were imported and 40 (40/77, 52%) were autochthonous. In 2024, 327 cases were investigated, 61 (61/327, 18%) were positive for DENV, 46 (46/61, 75%) were imported and 15 (15/61, 25%) were autochthonous. From the beginning of 2025 to date, 36 suspected cases of DENV have been examineted, resulting in 5 (5/36, 14%) imported confirmed cases. The 40 autochthonous cases in the 2023, were related to an outbreak in Lodi province (DENV1), whereas the 15 autochthonous cases in 2024 were connected to an outbreak in Brescia province (DENV1). The whole genome sequencing of a sub-set of DENV1 cases from the 2023 autochthonous outbreak revealed that the DENV1 sequences were phylogenetically related to DENV1(genotype V/D) strains from South America. While phylogenetic analysis conducted on the autochthonous 2024 cases showed correlation with DENV1(genotype III/A) strains from West Africa. The comparison of the sequences from both outbreaks showed no genetic and geographic correlation.

Conclusion: Virological surveillance proved to be crucial for the diagnosis of 143 DENV cases in the 2023-2025 period, allowing the interception of two autochthonous outbreaks of DENV1 in Lombardy: the first occurred in 2023 in Lodi province and the second in 2024 in Brescia province. The data from this study underscore the importance of DENV surveillance to monitor the introduction of new cases and the possible occurrence of autochthonous outbreaks.

330 - Parvovirus B19 Infection in Pregnancy: Clinical Manifestations and Fetus Outcome

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Parvovirus B19 Infection in Pregnancy: Clinical Manifestations and Fetus Outcome

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Introduction

Parvovirus B19 (B19V) belongs to the Parvoviridae family, genus Erythroparvovirus and can be transmitted vertically from mother to fetus during pregnancy. There is no antiviral therapy for B19V infection. Management of congenital B19V infection thus relies on secondary prevention of severe fetal consequences due to anemia. During the first months of 2024, we observed an upsurge of B19V infection, indicating an epidemic wave. The purpose of the present study is to collect epidemiological and clinical data of B19V infection in pregnancy from Obstetrics wards of major hospitals of the Lombardy region. Here we present data from Fondazione IRCCS Policlinico San Matteo, Pavia.

Materials and Methods

This is an observational study based on collection of clinical and virological data relevant to B19V infection in pregnant women and their fetuses/newborns occurring between 01/01/2024 and 31/12/2024.

Results

During 2024, 36 pregnant women with ascertained B19V infection were enrolled. The infection occurred at a median time of 16 weeks of gestation (range: 2-34). Symptoms occurred in 44.4% women and consisted in fever (47.0% cases), rash (41.1%) and arthralgia (35.3%). Exposure to individuals with B19V infections was documented in 6 cases, and exposure to children was documented in 28 cases. All women were IgM positive at diagnosis, and 33 of them (94.3%) were also IgG positive. Median B19V DNA load was 47060 copies/ml plasma (range: 519-5790440). Ultrasound fetal abnormalities were observed in 6 cases at a median time of 16 weeks of gestation (range: 12-30) and consisted in intrauterine growth retard, pericardial effusion, thoracic effusion, ventriculomegaly, low percentile head circumference and anemia. Amniocentesis was performed in 2 cases (the one with IUGR and the one with ventriculomegaly) and resulted positive for B19V DNA. Funicolocentesis was perforemd in the fetus with anemia and resulted positive for B19V DNA. This fetus received intrauterine transfusion and resolved anemia. No pregnancy was terminated and all the newborns were alive and well at birth. The clinical follow-up of infants is ongoing.

Discussion and Conclusions

This data will be completed those collected from the other centers participating in the study. Prompt treatment of anemia was able to resolve the pathology in symptomatic fetuses. A policy of serological screening and follow-up in pregnant women during the epidemic periods could help in rapid identification of cases of B19V infection, and should be considered to improve management of fetal complications.

331 - Impact of CFTR mutations on SARS-CoV-2 receptor expression: unveiling host-pathogen interactions through human intestinal organoids.

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Impact of CFTR mutations on SARS-CoV-2 receptor expression: unveiling host-pathogen interactions through human intestinal organoids

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Introduction: Recent evidence indicates reduced SARS-CoV-2 replication in epithelial cells with CFTR mutations, correlating with lower infection rates and milder COVID-19 in cystic fibrosis (CF) individuals. While ACE2 expression differs between wild-type (WT) and CF cells, these changes do not fully explain altered viral susceptibility, suggesting that CFTR function and expression may play a role, underscoring a broader role for CFTR in host-pathogen interactions at the airway surface. To do so, we used human intestinal organoids from healthy donors and CF individuals carrying the F508del/F508del and W1282X/W1282X CFTR mutations to assess SARS-CoV-2 susceptibility in relation to CFTR expression and function. CFTR modulators were also used to confirm the direct impact of CFTR expression/function.

Materials and Methods: WT, F508del/F508del, and W1282X/W1282X CFTR rectal organoids were embedded in Matrigel and cultured in expansion medium. F508del/F508del CFTR organoids were treated for 24h with CFTR correctors VX-661 (3 μM) and VX-445 (3 μM), while W1282X/W1282X organoids were treated for 24h with CC-90009 (1 μM), a cereblon (CRBN) E3 ubiquitin ligase modulator known to restore CFTR readthrough. Basal CFTR, ACE2, and TMPRSS2 gene expressions were assessed by Real-Time PCR in organoids harboring different CFTR mutations, with and without treatments, to investigate the relationship between CFTR expression/function and SARS-CoV-2 susceptibility.

Results: CFTR gene expression was reduced in F508del/F508del and W1282X/W1282X organoids compared to WT, with partial restoration following ETI or CC-90009 treatment, respectively. ACE2 and TMPRSS2 expressions were higher in F508del/F508del and lower in W1282X/W1282X compared to WT. Both variants showed reduced ACE2 and TMPRSS2 expression after CFTR modulator treatment.

Discussion and Conclusions: We previously observed higher SARS-CoV-2 infection rates in WT epithelial cells compared to CF, with infection levels increasing following treatment with CFTR modulators. In the present study, our data from CF organoid model indicate that CFTR modulator treatment reduces the expression of the major SARS-CoV-2 receptors ACE2 and TMPRSS2 to levels comparable to those found in WT. This observation may suggest a comparable pattern of viral infection and permissiveness, potentially independent of receptor expression alone. Other intrinsic factors possibly modulated by functional CFTR, such as ion transport regulation, epithelial barrier integrity, or intracellular signaling pathways, may influence cellular susceptibility to SARS-CoV-2. Further direct infection experiments are required to validate this hypothesis and elucidate the underlying molecular mechanisms.

332 - Klebsiella spp. Carbapenemase VIM producers: evaluation of the activity of bacteriophage KPN2177 alone and in synergy with meropenem

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***Klebsiella* spp. Carbapenemase VIM producers: evaluation of the activity of bacteriophage KPN2177 alone and in synergy with meropenem**

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Introduction: *Klebsiella* spp. are commensals of the human microbiota and a leading cause of opportunistic nosocomial infections. The incidence of multidrug-resistant (MDR) strains of *Klebsiella pneumoniae* causing serious infections is increasing, and *Klebsiella oxytoca* is an emerging pathogen. Bacteriophages (phages) are viruses that can infect and kill bacteria. This study aims to evaluate the activity of phage KPN2177 in *K. pneumoniae* and K. *oxytoca* carbapenemase VIM producer strains alone and in combination with the antibiotic meropenem.

Materials and Methods: Phage KPN2177 was extracted from the wastewater of the Verona University hospitals, sequenced with Illumina and analysed with BV-BRC software (Unicyler assembly pipeline). Two *K. pneumoniae* and one *K. oxytoca* VIM producer clinical isolates were considered in this study. Plaque assay was performed for all strains. The phage activity was evaluated with the Plaque Forming Unit (PFU) assay. Phage kinetics curves, followed with Glomax Discover instrument (Promega) at 600nm with measurements every 30 min for 24h, were performed using different antibiotic concentrations and a phage concentration of 10^{-7}

PFU/ml. Four tests were running at the same time: strain alone, strain plus meropenem, strain plus phage and strain plus meropenem and phage.

Results: KPN2177 sequencing showed a lytic phage of about 50kb, with 85 proteins of which 42 were characterised. All three strains were categorised as susceptible (S) considering the susceptibility up to the 10^{-7} dilution and above. Analysis of kinetic curves evidenced that the phage KPN2177 showed good lytic activity alone and good synergy profiles, especially in the long term (24 h), where regrowth of the strain is seen in the phage-only condition but not in the combined condition with meropenem.

Conclusions: We can assess that the combination of the phage KPN2177 and meropenem showed a synergic effect in the *K. pneumoniae* and *K. oxytoca* VIM producers under investigation. Note of worth is the continued monitoring of the curves for a long period, showing how critical it was to visualise the synergy over a long time. Analysis of *Klebsiella* spp strains with the phage KPN2177 in combination with other antibiotics besides meropenem is under study.

341 - Antimicrobial Properties and Potential Applications of Pseudomonas gessardii M15 rhamnolipids

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Antimicrobial Properties and Potential Applications of *Pseudomonas gessardii* M15 rhamnolipids

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Introduction: The continuous outbreak of drug-resistant bacterial and viral infections imposes the need to search for new drug candidates. The cell membrane has emerged as a promising new therapeutic target, and lipophilic molecules, such as biosurfactants, are currently being utilized. Biosurfactants are amphiphilic compounds produced by microorganisms that are able to lower the surface tension between two phases. Rhamnolipids (RLs) are biosurfactants mainly produced by the *Pseudomonas* genus, possessing a huge range of different bioactivities, such as antimicrobial, antifungal, and antibiofilm. One of the main concerns with these molecules is the pathogenicity of their main producer, *P. aeruginosa*, an opportunistic pathogen that may seriously affect human life. To overcome this issue, we evaluated the antimicrobial activity of a rhamnolipids mixture produced by the non-pathogenic Antarctic marine bacterium Pseudomonas gessardii M15 in this work.

Materials and Methods: *Pseudomonas gessardii* M15 was isolated from Antarctic marine sediment, and its RLs mixture (M15RL) was characterized by mass spectrometry. The antimicrobial activity of M15RL was investigated against a panel of bacterial *Bacillus cereus*, *Listeria monocytogenes*, clinically isolated *Staphylococcus aureus* strains, herpes simplex type 1, type 2, human coronavirus 229E, OC43, and SARS-CoV-2. We also evaluated the

antimicrobial activity of M15RL on functionalized cotton swabs against S. aureus and plastic surfaces against HSV-1 and HCoV-229E. Finally, we assessed the cytotoxicity and ability of the mixture to resist the harsh conditions (pH and temperature).

Results: Mass spectrometry revealed the presence of a complex mixture of mono-RLs, with some new congeners. M15RL exhibited bactericidal and virucidal activities in the 6 to 25 µg/mL range. No cytotoxicity and hemolysis were observed in the activity range. Finally, M15RL was successfully used to functionalize cotton swabs and plastic surfaces, as proofs of concept in applications for wound dressing and surface disinfectants against MRSA and enveloped viruses.

Discussion and Conclusions: The results highlighted the high biotechnological potential of this mixture, suggesting the opportunity of using it as an antiviral additive in formulations of hand sanitizers, surface and household cleaners, and as antibacterial agents, functionalizing surfaces and materials for wound dressing and food packaging.

348 - The impact of blood culture incubation time on the diagnosis of bloodstream infections: a systematic review

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The impact of blood culture incubation time on the diagnosis of bloodstream infections: a systematic review

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Bloodstream infections (BSIs) represent a significant global public health challenge. Advances in microbiological diagnosis rely primarily on the detection of blood culture (BC) positivity through automated incubators. The duration of blood culture incubation is crucial for optimizing the diagnosis of bloodstream infections, with protocols commonly set at 5 or 7 days to maximize pathogen recovery. Although different protocols are used across microbiology laboratories, no consensus guidelines currently exist regarding the optimal incubation time. Nevertheless, most blood cultures become positive within 48 to 72 hours. To determine the optimal BC incubation period, we conducted a systematic review and meta-analysis. Following PRISMA recommendations, studies published between January 2000 and May 2024 were retrieved from Medline, Embase, and Web of Science databases, with no language restrictions. The protocol, based on the PICO methodology, compared standard 5-day incubation with both shorter (4-day) and prolonged (>5-day) protocols. BC positivity rates (excluding contaminants) were analyzed per set or per bottle, and for specific infections such as BSIs caused by *Brucella* spp. and infective endocarditis. Study quality was assessed using the Newcastle-Ottawa Scale. Out of 4,740 studies identified, 11 were included. Risk of bias was low in 8 studies and moderate in 3. Of the included studies, 10 were retrospective observational studies and 1 was prospective. The meta-analysis showed that 4-day incubation significantly reduced BC positivity compared to the 5-day protocol (4 studies; odds ratio [OR] 0.65, 95% confidence interval [CI] 0.56–0.74). Conversely, prolonged incubation (>5 days) increased the number of

positive BC bottles (4 studies; OR 1.36, 95% CI 1.11–1.66) and sets (3 studies; OR 1.29, 95% CI 1.12–1.49). As shown in the subgroup analysis, prolonged incubation significantly enhanced the detection of BSIs caused by *Brucella* spp. (OR 49.09, 95% CI 7.13–338.14), and probably also of those associated with infective endocarditis (OR 1.74, 95% CI 1.00–3.03). Despite subgroup analyses, heterogeneity remained high. However, when excluding from the analysis the subgroups of BSIs caused by *Brucella* spp. and infective endocarditis, the meta-analysis showed a non-significant increase in the number of positive BC bottles (2 studies; OR 1.16, 95% CI 0.94–1.42). A 5-day incubation protocol provides an effective balance for diagnosing most bloodstream infections, ensuring accurate pathogen recovery. However, prolonged incubation should be preferred when *Brucella* spp. infection or infective endocarditis is suspected. Further research is needed to define evidence-based guidelines on the optimal duration of blood culture incubation.

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356 - Development of a neutralization assay platform for the safe evaluation of neutralizing antibodies against arboviruses in human and animal samples

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Development of a neutralization assay platform for the safe evaluation of neutralizing antibodies against arboviruses in human and animal samples

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Introduction: The spread of flaviviruses like Dengue (DENV), Zika (ZIKV), West Nile (WNV), and Tick-borne Encephalitis (TBEV) into new temperate regions has increased significantly in recent years, driven by factors such as climate change, urbanization and global travel. These viruses are transmitted by arthropod vectors like mosquitoes and ticks, posing a serious public health threat to both humans and animals worldwide. Plaque reduction neutralization test (PRNT) is the gold standard for flavivirus serologic diagnosis but is performed using live infectious viruses in high biosafety level laboratories (BSL-3), raising safety and technical issues. To overcome these limitations, our objective is to implement a high-throughput, safe and versatile diagnostic pseudovirus platform based on non-replicating Single-Round Infectious Particles (SRIPs) expressing luciferase and pseudotyped with prME glycoproteins. SRIPs mimic the viral entry and early replication stages but are incapable of producing infectious progeny, allowing assays to be performed in biosafety level 2 (BSL-2) laboratories. **Materials and Methods:** To generate SRIPs, 293T Lenti-X cells were co-transfected with a yellow fever virus (YFV)-derived replicon plasmid encoding the luciferase reporter protein, a YFV capsid plasmid and plasmids encoding the prME glycoproteins from selected flaviviruses, including DENV, ZIKV, WNV and TBEV. The produced SRIPs were titrated on VERO cells and employed in a neutralization assay using sera from TBEV-infected goats and PRNT-positive sera from individuals infected with WNV, ZIKV, and DENV. Human and animal sera were serially diluted, incubated with SRIPs, and then applied to VERO cells. After 72 hours, neutralizing antibody (nAb) titers were expressed as the serum dilution that caused a 50% reduction in RLU (ID50) relative to cells treated with SRIPs alone. **Results:** Luciferase-expressing SRIPs pseudotyped with prME proteins from each flavivirus were successfully generated and exhibited high infectivity in VERO cells. The SRIP-based neutralization test (SRIP-NT) was exploited to assess the presence of nAbs in human PRNT-positive sera as well as sera from TBEV-infected goats. The results showed that the SRIP-NT

effectively detected antiviral neutralizing activity in both human and animal sera, consistent with the PRNT data. **Discussion and Conclusions:** After optimization and standardization, these platforms can be rapidly adapted, by substituting the prME glycoproteins with those of different flaviviruses. This innovative and versatile tool will enable the rapid and safe quantification of antiviral nAbs and the simultaneous screening of multiple sera against different viruses, which could support the tracing and surveillance of emerging and re-emerging infections.

362 - Monoclonal antibodies as appealing therapeutics and prophylactic tools for broad-spectrum viral infections.

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Monoclonal antibodies as appealing therapeutics and prophylactic tools for broad-spectrum viral infections.

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Abstract

The emergence of novel viral pathogens with pandemic potential is one of the most significant threats to global public health in the 21st century. Recent outbreaks, including the SARS-CoV-2-caused pandemic of 2020, monkeypox surge, and recurring influenza threats, have emphasised the urgent need for robust strategies to prepare for viral threats. These events have revealed significant shortcomings in our therapeutic options and underlined the

importance of developing broad-spectrum antivirals. Viruses with pandemic potential share several characteristics that make them particularly concerning: high transmissibility, ability to cross species barriers, capacity for rapid mutation, and potential for severe clinical outcomes. The recent global mpox outbreak demonstrated how quickly these pathogens can spread across international borders, affecting vulnerable populations and straining healthcare systems worldwide.

Monoclonal antibodies (mAbs) have emerged as one of the most promising therapeutic approaches in the fight against viral infections, offering unique advantages in terms of both treatment and prevention. Unlike traditional small-molecule antivirals, which target specific viral proteins and are susceptible to resistance mutations, mAbs can provide broad-spectrum activity by recognising conserved epitopes across multiple viral strains and species.

We characterized an anti-IFITM2 mAb able to interfere with spike-mediated internalization of SARS-CoV-2 in host cells and investigated, in cell-based assays, its potential antiviral activity against a broad spectrum of highly pathogenic RNA and DNA viruses, including MpoxVirus. The anti-IFITM2 mAb was able to broadly reduce viral replication of different respiratory viruses (Influenza A, H3N2, H1N1, Respiratory Syncytial Virus) in a dose-dependent manner, and against Human Herpesvirus 1, suggesting that the IFITM2-mediated mechanism of host cell invasion may be shared with viruses other than SARS-CoV-2.

Targeting IFITM2 may be a more robust and broadly applicable approach to inhibiting viruses that bind to and enter host cells via IFITM2-associated plasma membrane docking mechanisms.

06 6. Genoma e mobiloma

28 - Human Endogenous Retrovirus K as possible novel biomarker and therapeutic target in colorectal cancer

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Human Endogenous Retrovirus K as possible novel biomarker and therapeutic target in colorectal cancer

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Introduction: Human Endogenous Retroviruses (HERV) account for approximately 8% of the human genome, where they are integrated and typically remain silent. Despite their inactivation, numerous retroviral sequences retain intact open reading frames (ORFs) capable of producing retroviral transcripts and/or proteins, which have been detected in colorectal cancer.
Material and Methods: Fifty-eight patients with colorectal cancer were enrolled (ASST Papa Giovanni XXIII Hospital, Bergamo, Italy). Expression of HERV-H, -K, -P, -R *env* genes, and HERV-K *pol, gag, rec* and *np9* genes were analysed in tumor tissues, adjacent healthy tissues and, when possible, in peripheral blood. Associations among clinical characteristics, such as tumor stage, location, loss of function of DNA repair genes, and HERV gene expression levels were analysed. Expression of HERV-K Env and Pol protein was evaluated in tumor and healthy tissue of 11 patients by Western Blot. Presence of HERV transcripts was investigated in serum-extracellular vesicles (EV).

Results: All transcripts, but HERV-K *rec,* were more expressed in blood compared to tissue samples; HERV-K gag exhibited a greater fold increase in tumor tissue relative to healthy tissue compared to all other HERV genes ($p < 0.001$). HERV-K *pol* expression in the blood samples was associated with lymphovascular invasion, stage pTNM III of the tumor, and was also positively correlated with the number of tumor-positive lymph nodes (Spearman's $rs=0.44$, $p=0.01$). Conversely, HERV-K *env* expression in tumor samples was associated with the retained status of the DNA repair genes PMS2 and MLH1 ($p<0.05$). Higher translation of HERV-K Env and Pol proteins were observed in adjacent healthy tissue compared to the nearby tumor sample, according to the western blot analysis. HERV transcripts were detected in EV, ranging from 5.5% (-K *rec*), and 100% (-K *np9*) of tested sera.

Discussion and Conclusions: Among all the tested HERV, HERV-K genes showed differential expression in the patients' clinical samples, which could be useful for the definition of new serum biomarkers (i.e. HERV-K *pol*) and therapeutic targets (i.e. HERV-K *gag*). Higher expression of the HERV-K proteins in the adjacent healthy tissues might be representative of a "hit and run" mechanism to promote tumorigenesis, while HERV sequences in serum-EV might be transferred from one cell to another, favouring cellular transformation.

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29 - Accurate differentiation of Burkholderia cepacia complex species: a pangenome-based approach for their rapid identification

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Accurate differentiation of *Burkholderia cepacia* complex species: a pangenome-based approach for their rapid identification

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Introduction - The *Burkholderia cepacia* complex (BCC) is a collection of closely-related opportunistic pathogenic bacteria, including more than 24 *Burkholderia* species. Members of the BCC are frequently associated with contamination of medical devices and with nosocomial infections, posing a significant threat to vulnerable subjects, such as immunocompromised individuals and patients with cystic fibrosis. The high phenotypic and genotypic similarity among BCC isolates makes their rapid identification challenging, as traditional methods fail to distinguish species within and outside the complex. These challenges, together with recent revisions in the taxonomic classification of the BCC species, highlight the need for reliable differentiation strategies of species within the complex, in order to guide appropriate clinical therapies and effectively monitor their epidemiological spread. Therefore, here we present our pangenome-based workflow for identifying potential discriminatory molecular markers for individual BCC species, leveraging all available genomes in public databases.

Materials and Methods - Based on genome quality and the reliability of their taxonomic classification, BCC genomes were first grouped into three major categories, each serving a specific role in the overall workflow. The most reliable and high-quality BCC genomes were involved in the analysis of conserved component of the BCC pangenome, called "core-genome", in order to assess its potential to discriminate between species within the complex. Then, we conducted a whole-genome-based investigation to identify species-specific genomic markers, namely sequences exclusively found in a single BCC species. These sequences were then used to design PCR primer pairs for each BCC species, whose selection was guided by their *in silico*-predicted discriminatory potential as well as by their genomic mapping location.

Results - Core-genome-based phylogenetic analysis revealed the existence of 25 distinct clusters reflecting individual BCC species. Multiple marker sequences were identified for each species from both the core and accessory components of the BCC pangenome, leading to the generation and *in silico* testing of species-specific sets of primers.

Discussion and Conclusions - Our workflow enabled a large-scale, whole-genome-based, and taxonomically updated pangenomic study of the BCC. Findings revealed that the BCC core-genome alone possesses discriminatory potential for all species of the complex, which could

be leveraged to identify species-specific markers and designing discriminatory PCR primer pairs. However, *in silico* tests suggest that the accuracy of species identification could be improved by using a multi-primer approach rather than single primers.

40 - Pangenomic analysis on Neisseria meningitidis serogroup W strains for identifying genomic regions involved in the pathogenesis

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Pangenomic analysis on *Neisseria meningitidis* serogroup W strains for identifying genomic regions involved in the pathogenesis

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Background
Invasive meningococcal disease (IMD) outbreaks associated with Hajj and Umrah pilgrimage events and travel related to the Kingdom of Saudi Arabia (KSA) are well recognized. Past outbreaks have been associated with the substantial intercontinental spread of serogroup W *Neisseria meningitidis* (NmW) strains belonging to the “Haji” strain sublineage.

This work aimed to provide some preliminary data on the pangenome structure of NmW “Haji” isolates, comparing with “non-Haji” isolates, identified in Italy, applying a hybrid assembling pipeline.

Materials and methods
A detailed genomic variability analysis was provided for NmW “Haji” isolates collected in Italy during the early months of 2025. In fact, the whole genome of one NmW “Haji” isolate was characterized at the pan genomic level, applying a hybrid genomic reconstruction approach based on Unicycler and using an "ad hoc" refining pipeline. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) allowed us to carry out an annotation step along the complete genome. Moreover, the phylogenetic relationship of three “Haji” and three “non-Haji” NmW isolates collected in Italy was investigated using BWA and IQTREE, comparing also with other hyperinvasive Nm strains.

Results

Firstly, a in depth whole genome reconstruction was carried out for one NmW “Haji” isolate linked to the Umrah pilgrimage in 2025, obtaining a complete genome of ~2.1 Mbp long. This genome was annotated, reporting 2,162 genes, of which 1,939 Coding Sequences (CDSs) with protein, 59 tRNAs 4 for each 5S, 16S, and 23S rRNAs. To better define the genomic differences between NmW “Haji” isolates and NmW “non-Haji” isolates, a phylogenetic analysis, based on SNPs along the whole genome, was carried out using the NmW “Haji” strain as a new reference.

Thus, this approach enabled us to emphasize, with high statistical confidence, the strong separation between NmW genomes “Haji” and “non-Haji” collected in Italy.

Conclusion

Despite the many advances in understanding IMD, much remains to be discovered about molecular mechanisms involved in pathogenesis. This work enables to understand the genomic scaffold of meningococcal NmW “Haji” strains, but also it might lead the way to identifying possible genomic regions specifically involved in the Nm pathogenesis using a pangenomic approach.

49 - BlpU, bacteriocin encoded by Streptococcus salivarius 24SMBc, is responsible for anti-pneumococcal activity

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BlpU, bacteriocin encoded *by Streptococcus salivarius* 24SMBc, is responsible for anti-pneumococcal activity

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Introduction: *Streptococcus salivarius* is part of the human oral microbiota that can produce strain-specific bacteriocins with different antimicrobial ability and spectrum of activity. The probiotic strain *S. salivarius* 24SMBc is known for its strong antimicrobial activity against *Streptococcus pneumoniae* and *Streptococcus pyogenes*. In this work, we evaluated the effect of disruption of class IIc bacteriocin genes *blpU* and *blpK* on the antimicrobial activity of 24SMBc and tested the bacteriocin BlpU against different serotypes of *S. pneumoniae*.

Material and Methods: Knockout mutants of bacteriocin genes (*ΔblpU*::*Spec*^{R}, *ΔblpK*::*cat*, *ΔblpU*::*Spec*^{R}-*ΔblpK*::*cat*) were constructed by natural transformation. The spectrum of antimicrobial activity of *S. salivarius* 24SMBc and its derivative strains was tested by spot-on-lawn assays against several streptococci and other pathogenic Gram-positive bacteria. Isolation of BlpU was achieved by acetone precipitation of the cell-free supernatant (CFS) of *S. salivarius* 24SMBc. Quantification of BlpU was performed by a modified spot-on-lawn assay, comparing the inhibition halo of CFS proteins with a synthetic form of BlpU at scalar dilutions. Similarly, MIC and MBC of synthetic BlpU was measured for different *S. pneumonia*e serotypes (NT, 9V, 15C, 19A, 19F).

Results: Our study demonstrated that *S. salivarius* 24SMBc exerts its antimicrobial activity against several streptococci, including *S. pneumoniae*, and other pathogenic bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*. This activity is mediated by the class IIc bacteriocin BlpU, as the strain *ΔblpU*::*Spec*^{R} and the double mutant *ΔblpU*::*Spec*^{R}-*ΔblpK*::*cat* showed a complete loss of activity. Active BlpU was recovered from the CFS, and its production reached a concentration of 38 mM in *S. salivarius* 24SMBc wild type. Furthermore, MIC values for BlpU (3.12-1.56 µM) and MBC (6.25-3.12 µM) confirmed the strong bactericidal activity against different *S. pneumoniae* serotypes.

Discussion and Conclusions: Class IIc bacteriocin BlpU plays the main role in bacteriocin-mediated antimicrobial activity of *S. salivarius* 24SMBc, with a wide spectrum of activity against several opportunistic pathogens of the Firmicutes phylum. Furthermore, even at low concentrations BlpU shows a strong bactericidal activity against *S. pneumoniae*, suggesting potential applications targeting this pathogen.

69 - A multiclonal outbreak of carbapenem-non-susceptible Pseudomonas aeruginosa in neonatal intensive care unit

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A multiclonal outbreak of carbapenem-non-susceptible Pseudomonas aeruginosa in neonatal intensive care unit

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Introduction: Pseudomonas aeruginosa (PSAE) can persist in moist environments in healthcare settings, representing a source for outbreaks of healthcare-associated infections. Here is a multiclonal outbreak of carbapenem-non-susceptible PSAE in the neonatal-intensive-care unit (NICU) of Fondazione IRCCS Policlinico, Milan, Italy.

Materials and Methods: Whole genome sequencing (WGS) was performed on PSAE strains consecutively isolated from neonates and the environment. Bacterial typing and molecular resistance mechanisms were inspected by MLST-tool and AMRfinder. SNPs were called by Snippy using PAO1 as reference. Antimicrobial susceptibility to carbapenem was tested by broth microdilution using the Microscan-WalkAway (Beckman, Coulter). Carbapenem-non-susceptible PSAE was defined with a minimal inhibitory concentration (MIC) to Meropenem (MEM) higher than 2 mg/L. Associations between SNPs and MEM-phenotypic resistance were tested through Mann-Whitney. Maximum Likelihood and Minimum Spanning Trees were used to determine strain correlations and identify potential transmission clusters (defined as bootstrap=100% and SNP distances <5).
Results: Between August and September 2024, two sepsis and four colonizations by carbapenem-non-susceptible PSAE were reported in 6 neonates in NICU. As a result, PSAE genomic surveillance was performed in all weekly collected clinical isolates and ad-hoc collected contaminated environment samples (total isolates: 18 clinical samples from 8 neonates and 12 sink-siphons samples from nine rooms). Phylogenetic analyses showed the circulation of four carbapenem-non-susceptible STs (ST446, ST395, ST253, ST179); notably, ST446, ST395, and ST253 harboured the mutations oprD-D30T and mexR-V126Q, both significantly associated with higher MEM MIC (P=0.0004). Twelve clinical isolates accounting for 6 patients (two developing sepsis) were organized in a ST446 large transmission chain (SNP-distance: 0). One clinical isolate belonging to ST179 was genetically related to one isolate detected in moist environments, both carrying the blaVIM gene plus the orpD-V359L. The other three carbapenem-non-susceptible clinical isolates belonged to the ST395 and ST253. The remaining 2 clinical and 11 environmental isolates were characterized by carbapenem-susceptible STs (ST155=2; ST210=1; ST244=1; ST309=3; ST235=4; ST674=2).
Discussion and Conclusions: Genomic characterization of PSAE in clinical and environmental isolates allowed us to define a multiclonal diversification of the carbapenem-non-susceptible PSAE outbreak in NICU, characterized by one transmission chain of clinical origin and one transmission pair involving a contaminated environment, and sharing in majority of cases mutations in oprD and mexR genes.

91 - Heteroresistance in a vancomycin variable Enterococcus faecium from marine sediment

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Could heteroresistance underlie the resistant phenotype in a vancomycin-variable *Enterococcus faecium* from marine sediment?

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Introduction: Vancomycin-variable enterococci (VVE) have emerged as hospital-acquired pathogens alongside vancomycin-resistant enterococci. VVE strains are susceptible to vancomycin but carry a silent *van* operon allowing the reversion to a resistant phenotype after vancomycin exposure, posing a risk of treatment failure. This study investigates the phenotypic and genetic features of *Enterococcus faecium* JSEG15, a VVE strain from marine sediment.
Materials and Methods: Whole-genome sequencing (WGS) was performed to genetically characterize the parental strain and three revertants selected through vancomycin exposure. RT-qPCR was used to assess the *vanA* copy number and expression levels in the VVE strain and its revertants. The *vanA* gene transferability was performed by filter-mating experiments. Vancomycin heteroresistance was evalueted by E-test and population analysis profiling.
Results: The *E. faecium* JSEG15 strain, belonging to ST80 (one of the dominant *E. faecium* sequence type associated with nosocomial infections), was susceptible to vancomycin despite carrying a Tn*1546*-like transposon on a 36,019 bp plasmid (pEG15). The strain showed a deletion in the promoter region of *vanHAX* cluster probably causing a constitutive low level *vanA* expression in the majority of cell population. However, a gradual exposure to increasing vancomycin concentrations led to the selection of three resistant revertants with growing levels of resistance. All revertants harbored a deleted Tn*1546*-like element on a 34,728 bp plasmid (pEG15rev). In the revertants, IS*1216* insertion caused a 1238-bp deletion removing the *vanY*3'-end and *vanZ* gene. Notably, JSEG15-rev2 and JSEG15-rev3 acquired an additional chromosomal copy of the *vanHAX* cluster, along with increased *vanA* copy number and expression, explaining their higher resistance levels. JSEG15 and JSEG15-rev1 showed inducible resistance to vancomycin whereas JSEG15-rev2 and JSEG15-rev3 exhibited

constitutive *vanA* expression. Only JSEG15-rev3 was able to transfer resistance by *in vitro* conjugation. Both the parental strain and revertants exhibited vancomycin heteroresistance, with revertants showing higher frequencies of resistant subpopulations (up to 10^{-3}–10^{-4} in JSEG15-rev3 vs 10^{-6} in JSEG15), likely due to selection of these subpopulations during vancomycin exposure.

Discussion and Conclusions: Heteroresistant/VVE strains pose a clinical challenge, as resistant subpopulations emerging during therapy may lead to treatment failure. To the best of our knowledge, this is the first report of a VVE isolate from marine sediment, indicating environmental dissemination of clinically relevant strains. Strengthened monitoring of coastal waters inputs is essential to safeguard environmental and human health.

92 - Novel transposons carrying cfr(B) and cfr(C) linezolid resistance genes in Clostridioides difficile isolates from calves, Italy

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Novel transposons carrying *cfr*(B) and *cfr*(C) linezolid resistance genes in *Clostridioides difficile* isolates from calves, Italy

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Introduction: *Clostridioides difficile*, a well-known nosocomial pathogen, is increasingly recognized as a potential zoonotic agent. Rising antimicrobial resistance, including to oxazolidinones like linezolid, is often driven by acquisition of transferable genes such as *cfr*. This study investigates three bovine *C. difficile* strains with high linezolid MICs to elucidate the genetic basis of the resistance.
Materials and Methods: Whole-Genome Sequencing (WGS) was used to comprehensively characterize *C. difficile* A501, A505 and A516 strains exhibiting high linezolid MICs (12-48 mg/L) and to clarify their phylogenetic relationships. Linezolid resistance genes transferability was assessed by filter mating experiments.

Results: WGS analysis revealed the presence of *cfr*(B) in the *C. difficile* A501 and A516 strains. These isolates were closely related and both belonged to the ST11 sequence type, a lineage known to colonize and infect livestock worldwide, as well as cause disease in humans, underscoring its zoonotic potential. In contrast, *cfr*(C) was identified in the *C. difficile* strain

A505, which belonged to the non-toxigenic ST15 clone, typically detected in both clinical and animal strains. The phylogenetic analysis showed that *C. difficile* A501 and A516 strains clustered with other toxigenic strains belonging to the ST11, while A505 grouped with non-toxigenic ST8 and ST15.

The *cfr*(B) gene was located on a novel 25,791-bp integrative and conjugative element (ICE), named ICE*Cd-cfr*(B), similar to an uncharacterized genomic region of *Clostridium* sp. C1, but significantly different from Tn*6218* transposon typically associated with this gene. The *cfr*(C) gene was found on a novel chromosomal 32,770-bp ICE, named ICE*Cd-cfr*(C), which was identical to an uncharacterized region of the *C. difficile* DSM 104450 chromosome. ICE*Cd-cfr*(C) exhibited high nucleotide identity but low coverage with a *cfr*(C)-carrying region previously detected in *C. perfringens* 19HBBNCP of calf origin from China (accession no. CP073070) and in the human *C. difficile* 020482 (accession no. CP028525.1) from USA. However, in *C. difficile* A505 the *cfr*(C)-containing region identified in *C. perfringens* and *C. difficile*, was splitted into two parts due to an 16,744-bp DNA insertion. Conjugation assays failed to demonstrate the transferability of *cfr*(B) and *cfr*(C) genes.

Discussion and Conclusions: To the best of our knowledge, this is the first report of *C. difficile* isolates from calves exhibiting high linezolid MICs due to novel *cfr*(B)- and *cfr*(C)-carrying ICEs. *C. difficile* animal isolates, belonging to ST11 and ST15 clones with zoonotic potential, could act as reservoir for the spread of linezolid resistance genes to human intestinal pathogens, posing a potential threat to public health.

94 - HDV sub-genotype 1 is characterized by increased genetic variability, which can promote the emergence of divergent genetic pathways that modulate HDV replicative potential and cytolytic activity

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HDV sub-genotype 1 is characterized by increased genetic variability, which can promote the emergence of divergent genetic pathways that modulate HDV replicative potential and cytolytic activity

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Introduction: Current knowledge on the extent of genetic diversification within the Hepatitis D Virus (HDV) ribozyme and the Hepatitis D Antigen (HDAg) domains (containing cytotoxic T lymphocyte [CTLE]) remains limited, as does understanding of their association with virological and biochemical markers. **Materials and Methods:** 103 full-length HDV sequences were obtained by Illumina (median [IQR] reads/seq: 62045[30460-91899]). Sub-genotypes, HDAg domains and CTLE (N=18) were defined according to Pascarella 2010 and Kohsar 2021. Amino acid (aa) residues were defined conserved if not mutated in 99% of sequences. **Results:** Individuals were mostly males with a median age of 54 (44-60) years, mainly from Eastern Europe (EE, 51%) and Italy (IT, 44.8%). Serum HDV-RNA and ALT were 5.6 (4.9-6.2)logIU/ml and 94 (65-152)U/L. Sub-genotypes 1c and 1e were the most prevalent (47.1% and 45.2%): 1c predominated in individuals from EE(77.6% vs 22.4%, P<0.001) while 1e in IT(77.5% vs 22.5%, P<0.001). HDV ribozyme was characterised by a 100% nucleotide conservation, while for HDAg nucleotide dropped to 62.8%. The degree of aminoacidic conservation within HDAg ranges from 42.3% in RNA binding domain (RDB)1 to 72.7% RDB2. The degree of genetic conservation of CTLEs ranges from 44.4% in CTLEs 1, 9, 12 to 87.5% in CTLE 9. Notably, CTLE 5 from individuals with HDV-RNA >5logIU/ml (70% of conserved aa with vs 20% without HDV-RNA <5 logIU/ml, P=0.025) showed lower genetic conservation, suggesting that an enrichment of mutations in this CTLE can enhance viral replication. Finally, despite a comparable degree of genetic conservation between sub-genotypes 1e and 1c, they were characterized by divergent genetic pathways. In particular, sub-genotype 1c was significantly associated with the selection of 7 specific mutations (I16T/V, N22S, D47E, R112K, T180A, A202S, prevalence ranging from 26.5% to 44.9% vs 0% in 1e, P<0.001). Conversely, sub-genotype 1e was significantly associated with the selection of 6 specific mutations (D29E, D46E, K113R, R131K, M171L, I188V prevalence ranging from 23.9% to 43.5% vs 0% in 1c, P<0.009). Notably, in sub-genotype 1c, the co-presence of I16V/T+D47E+A202S correlated with ALT>3ULN (100% vs 27.5%, P=0.001). **Discussion and Conclusion:** HDV sub-genotype 1 genetic diversification within HDAg functional domains and CTLEs, leads to the emergence of distinct mutational signatures that may impair immune recognition and enhance viral replication. Moreover, the co-presence of specific mutations can potentially contribute to liver inflammation. Therefore, the role of the high degree of genetic variability in affecting the proper HDV detection by the currently available diagnostic assays deserves further investigation.

96 - Whole-Genome Sequencing of carbapenem-resistant Escherichia coli from clinical samples, EURGen-CCRE survey, Italy 2019

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Whole-Genome Sequencing of carbapenem-resistant *Escherichia coli* from clinical samples, EURGen-CCRE survey, Italy 2019

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Introduction: The emergence of carbapenem-resistant (CR)-*Escherichia coli* (EC) represents a serious threat for public health worldwide. In Italy, the proportion of invasive CREC isolates has so far been very low, only 0.4% in 2024, as reported by the national antibiotic-resistance surveillance system AR-ISS. To gain genomic information to identify high-risk clones and obtain a more detailed understanding of transmission pathways, Italy participated in the carbapenem- and/or colistin-resistant *Enterobacterale*s (EURGen-CCRE) survey launched in 2019 by the European Centre for Disease Prevention and Control.

Materials and Methods: From May to October 2019, 17 large acute-care hospitals in Italy collected and sent to ISS a total of 61 consecutive, non-duplicated *E. coli:* 17 CREC and 44 carbapenem-susceptible (CS) *E. coli* (CSEC, comparator isolates). Susceptibility to carbapenems was confirmed at ISS by the reference broth microdilution method. WGS was performed using Illumina technology. MLST, resistome and phylogeny were investigated.

Results: Out of 61 *E. coli,* 17 CREC were mainly obtained from urine (41.2%) and blood (23.5%), **while** 44 **CSEC were** mainly from blood (43.2%) and urine (38.6%). CR**EC** were all resistant to at least one carbapenem (meropenem or ertapenem), while all the 44 CS**EC** were confirmed susceptible to carbapenems. CR**EC** belonged to 8 different STs, with the predominant ST131 (58.8%). CS**EC** were grouped into 26 STs, with ST131 (25%) and ST69 (11.4%) being the most frequent. All the CREC carried **1** carbapenemase gene: bla_{KPC-3} (10 isolates), bla_{KPC-2} (3), bla_{NDM-5} (2), bla_{VIM-1} and $bla_{OXA-181}$ (1 each). Extended-spectrum β-lactamase (ESBL) genes were found in most CREC (11/17, 64.7%): $bla_{CTX-M15}$ was the most frequent (7/17, 41.2%) gene, most reported in association with ST131 and bla_{KPC}. Five isolates harbored bla_{AmpC}. No mobile colistin resistance (*mcr*) gene was detected. CS**EC** had several ESBL genes: $bla_{CTX-M-15}$ (7/44, 15.9%, found in association with ST131), and bla_{AmpC} (16/44, 36.4%) were the most frequent. No *mcr* gene was detected. A core genome SNP-phylogenetic tree showed high diversity among the 61 *E. coli* isolates, with no major clades observed except for the branch including the ST131 isolates. **Of** these, 2 pairs of isolates had SNP differences **of** 0 **or** 1, respectively.

Discussion and conclusions: CR**EC** showed heterogeneous genetic background **with** ST131 the most frequent lineage found, mostly **carrying** $bla_{CTX-M15}$ and bla_{KPC}. Notably, bla_{NDM-5} along with other carbapenemase genes were detected. WGS should be strongly implemented at

national level for surveillance purpose **to obtain a deeper characterization, to** monitor and prevent the emergence and spread of high-risk clones causing difficult-to-treat infections.

100 - Genomic analysis of vancomycin-resistant Enterococcus faecium isolated from human infections and colonization in one hospital in Northern Italy, 2023-2024.

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Genomic analysis of vancomycin-resistant *Enterococcus faecium* isolated from human infections and colonization in one hospital in Northern Italy, 2023-2024.

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Introduction Vancomycin-resistant *Enterococcus faecium* (VREfm) causes a wide range of hospital infections especially in critically ill patients. In Italy, over the last decade, a worrisome increasing trend in the percentage of invasive VREfm was observed, reaching the rate of 32.5% in 2023, one of the highest in Europe. We used WGS approach to characterize VREfm collected over one year from one hospital in Lombardy, Northern Italy.

Materials and methods From March 2023 to March 2024, 116 VREfm from human infections and colonization, and 1 isolate from environment were obtained from one hospital in Lombardy and sent to ISS to be characterized by WGS using an Illumina platform. MLST, resistome and phylogeny were investigated.

Results Preliminary WGS analysis was conducted on 34 selected VREfm, of which 12 obtained from infections (3 blood, 1 CSF, 5 urine, 3 other samples), 21 from rectal swabs and 1 from environmental sample. MLST analysis identified 5 different sequence types (ST)s, being ST80 (76.5%) the most prevalent followed by ST1478 (11.8%). Resistome analysis showed that 33 VREfm shared the *vanHAX* genes and 1 carried both *vanHAX* and *vanHBX* genes. All isolates carried the genes conferring resistance to aminoglycosides [*aac(6')-Ii*] and to macrolides/streptogramins [*msr*(C)]; 97% (33/34) of isolates carried the macrolide resistant gene *erm*(B). All isolates showed known mutations in the *gyrA* and *parC* genes, conferring resistance to quinolones. In a minority of VREfm other resistance genes were found: 10/34 (29.4%) carried tetracycline resistance genes being *tet*(L) the most frequent (90.0%); 9/34

(26.4%) carried trimethoprim resistance gene *dfrG*; 8/34 (23.5%) were resistant to linezolid, of which 7 (20.6%) showed co-existence of transferable linezolid resistance genes *cfr*(D) and *poxtA* while 1 (3.0%) carried the 23S rRNA gene mutation. Out of 34 VREfm, 22 segregated into 6 different clusters (named C1-C6) with a SNP variation ranging from 0-1 (C3) to 0-19 (C5); the largest clusters (C4-C5) included 6 strains. Five clusters (C1-C5) included 20 VREfm belonging to ST80 while 1 cluster (C6) contained 2 isolates belonging to ST1478. Notably, C1 included 1 VREfm from environment and 1 from rectal swab. Among **7** VREfm with acquired resistance to linezolid, 6 belonged to C4.

Discussion and conclusions Preliminary analysis showed ST80 as the predominant VREfm circulating lineage. All VREfm were multiresistant with some carrying transferable linezolid resistance, raising concerns about intra- and inter-hospital spread and treatment implications. Continuous genomic surveillance is crucial for monitoring VREfm molecular epidemiology, supporting outbreak investigation and straightening antibiotic-resistance prevention and control within healthcare settings.

114 - Isolation of a Escherichia coli K12 conjugative-negative strain

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Isolation of a *Escherichia coli* K12 conjugative-negative strain

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Introduction

Conjugation, transformation, transduction, transposition, genetic transfer agent (GTA), and phagocytosis are the main mechanisms to transfer genetic materials among bacteria. Conjugation is the most evolved system that enables the transfer of many daltons of DNA including the entire chromosome from a donor to a recipient cell mediated by a conjugative plasmid. Since during conjugation donor and recipient cell do not divide, we hypothesised that conjugative plasmid produce a protein capable to arrest cell division, this protein is degraded by a protease at the end of the process. We reported here the selection of a mutant that continue to divide during conjugation.

Materials and Methods

Conjugation was carried out by standard method. Some tests were performed using a protease-negative mutant (*lon*) as recipient strain. To search for a spontaneous conjugative-negative mutant a FlacTn*10* episome was mated with a streptomycin resistant lactose negative-strain.

Results

Matig tests with *lon* mutants showed that the number of recombinants was reduced by more the 99,99% in comparison with the control showed. In other tests recombinants were selected as lactose-negative strains on McConkey agar containing streptomycin and tetracycline. In the mutant we hypothesized that dividing cell can receive F*lac,* but this episome was lost during cell division and the transposon Tn*10* was acquired by bacterial chromosome. Six colonies with these traits were found and they were successively mated with a donor strain and the number of recombinants was reduced by 90% in comparison with the control. Since F factor possess a genetic information coding for a protein targeting the gyrase gene and bacterial cells is killed if plasmid is lost, we hypothesized that the same target (gyrase) is used by F plasmid to stop division during conjugation Thus we transferred the gyrase gene from wild- type strain to a mutant and recombinants were found to able to conjugate.

Conclusion

The plasmid F produces a protein that can inhibit the gyrase gene during conjugation.

116 - Characterization of ESKAPE pathogens in 3D microbiota-mimicking system: insights from in vitro models

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Characterization of ESKAPE pathogens in 3D microbiota-mimicking system: insights from *in vitro* models.

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1. Introduction. The human microbiota plays a pivotal role in health and disease, driving growing interest from the pharmaceutical industry in microbiome-based therapeutics. This interest has highlighted the need for advanced in vitro models that closely mimic the human

microbial environment to boost therapeutic development. Bac^{3}Gel® is an advanced three-dimensional (3D) material designed for microbial culture, inspired by the biochemical and biophysical properties of human mucus. Its structure incorporates micro-gradients that replicate the heterogeneous environments colonized by bacteria within the human body, offering a powerful tool for studying host–pathogen interactions.

2. Materials and Methods. To assess Bac^{3}Gel® as an *in vitro* infection model, six high-priority pathogens known collectively as the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter cloacae*) were cultured on Bac^{3}Gel® and under planktonic conditions for 24 and 48 hours. Bacterial viability and colonization were evaluated using spot plating techniques. Confocal laser scanning microscopy (CLSM) was employed to visualize biofilm architecture and quantify live/dead bacteria within the 3D matrix.

3. Results. All ESKAPE pathogens successfully colonized and grew in Bac^{3}Gel®, with growth levels comparable to or greater than those in planktonic cultures. Spot plating confirmed viable populations at both times. CLSM analysis revealed well-structured biofilms, predominantly composed of live bacteria, which supports Bac^{3}Gel®'s ability to replicate in vivo-like infection dynamics. The inclusion of relevant micro-gradients appears to support realistic microbial behavior and tissue-specific colonization patterns.

4. Discussion and conclusions. Bac^{3}Gel® offers a reproducible, high throughput, human-relevant tool for cultivating pathogenic bacteria under conditions that simulate human mucosal environments. Its ability to replicate relevant gradients likely contributed to the observed microbial behaviors, offering a more accurate representation for studying microbial pathogenesis, biofilm development, and host–microbe interactions. These findings support Bac^{3}Gel® as a valuable model in microbiome-related research and can greatly aid the development of targeted, personalized therapeutic approaches.

136 - Interspecific horizontal transfer of IncA plasmid as a potential mechanism for blaVIM-1 dissemination in Enterobacterales

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Interspecific horizontal transfer of IncA plasmid as a potential mechanism for *bla*VIM-1 dissemination in *Enterobacterales*

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Introduction. The spread of carbapenemase-producing *Enterobacterales* (CP-CRE) is one of the most clinical issues for modern medicine since carbapenems are last-resort drugs to treat many severe infections. A key role in the dissemination of carbapenemase genes is played by conjugative plasmids, differing in host range and transfer efficiency. The reemergence of the bla_{VIM} gene associated with IncA-type plasmids has recently been reported. This study focused on a collection of VIM-producing CP-CRE isolates from different clinical samples of patients at "Ospedali Riuniti" of Ancona, to investigate in detail their resistome and the plasmids transfer efficiency.

Materials and Methods. 5 VIM-producing *Enterobacter cloacae* complex (ECC) and 5 VIM-producing *Klebsiella pneumoniae* (Kpn) were collected in 2021-23. All strains were analysed for their phenotypic and genotypic resistance properties and typed by XbaI-PFGE. WGS was performed by Illumina MiSeq and Nanopore MinION technologies. The sequences were analysed by tools available at the Center for genomic epidemiology (Resfinder, PlasmidFinder, MLST, CSI Phylogeny 1.4). Conjugation transfer assays were carried out using different VIM-

producing ECC and Kpn strains as donors and three different *Escherichia coli* strains (J53 Az^R, ATCC 25922 and a ST131 selected clinical isolate) as recipients.

Results. All strains were fully resistant to carbapenems and cephalosporins. Furthermore, all strains but one (ECC13) were aztreonam-resistant. The bla_{VIM-1} gene, contained in a class 1 integron, was carried by IncA plasmids in all strains except ECC13 which had an IncN plasmid. Moreover, a bla_{SHV-12} (related to aztreonam resistance) was carried by all IncA plasmids. Sequencing results show that ECC and Kpn strains share the almost identical IncA plasmid with a 99% DNA identity. ECC11 (VIM-IncA-ST286) and Kpn3 (VIM-IncA-ST39) were chosen as donors for conjugation assays. While no transconjugants were obtained from ECC13 (VIM-IncN-ST45), IncA plasmid from ECC11 and Kpn3 was successful transferred to all recipients used, with very different conjugation frequencies. In particular, the best transfer efficiency was obtained using *E. coli* ST131 recipient.

Discussion and Conclusion. In all but one CP-CRE strains recovered in the period 2021-2023, the bla_{VIM-1} was associated with the IncA plasmid, that also carried a bla_{SHV-12} and many other resistance genes. Our conjugation results confirmed the well-known broad-host-range of IncA. Furthermore, the different conjugation frequencies, obtained with three distinct strains, seems to highlight the relevant role of recipient strain in mating stability.

162 - Genetic diversity of Akkermansia bacteria in humans and other animals

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Genetic diversity of Akkermansia bacteria in humans and other animals

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Abstract

Introduction. *The mucin-degrading bacterium Akkermansia muciniphila has attracted enormous interest for its beneficial effects on human health. However, growing evidence suggests that the Akkermansia genus is populated by several species that differ in phenotypic characteristics and association with human traits. We present the most comprehensive phylotaxonomic analysis of Akkermansia genomes in terms of sample size and host representation.*

Materials and Methods. *We analyzed 1626 Akkermansia genomes, which represent the most comprehensive collection in terms of sample size and host representation. We applied approaches based on average nucleotide identities and on the biological species concept to characterized the Akkermansia genus.* A principal component analysis of accessory gene content was applyed to analyze species-specific or diet-specific association. *Finally, we applied population structure analysis A. muciniphila, the species that has been more intensely studied for its effects on human health.*

Results. *We show that the Akkermansia genus comprises at least 31 species, 13 of which can be detected in humans. T*he largest species diversity is contributed by non-human and non-mouse animals, and limited evidence of species-specificity is evident, with several

Akkermansia species detected in phylogenetically distant animals. Analysis of accessory gene content among species also failed to reveal species-specific or diet-specific associations, but rather reflected genome size. *Finally, investigation of the population structure of A. muciniphila, the species that has been more intensely investigated due to its effects on human health, clearly distinguished two phylogroups. However, analysis of laboratory mouse-derived genomes revealed that additional populations, specific to these animals, exist. Such populations show limited evidence of admixture, suggesting bottleneck or competition effects.*

Discussion and Conclusions. *Our data support* the concept that the genetic diversity of *Akkermansia* should be taken into account in experimental settings. They also call for sequencing efforts to characterize the wider genetic diversity of *Akkermansia* bacteria. Moreover, our findings are relevant to the translatability of the mouse model to human *Akkermansia* research.

174 - Circulation of different OXA-23-Acinetobacter baumannii lineages in the ASST of Pavia

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Circulation of different OXA-23-*Acinetobacter baumannii* lineages in the ASST of Pavia

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Introduction: The dissemination of MDR and carbapenemase-producing *Acinetobacter baumannii* (CRAB) is emerging in territorial facilities. We performed a molecular characterisation of OXA-23-producing *A. baumannii* (OXA-23-AB) isolates from the ASST of Pavia, which includes the Stradella, Varzi and Voghera Hospitals. Materials and methods: From February 2023 to May 2024, 50 CRAB have been isolated. Identification and antibiotic susceptibility profiles were obtained by BD Phoenix M50 and MicroScan AutoSCAN-4. Identification of resistance genes was performed by HybriSpot 12 PCR AUTO system on 12/24 strains. Typing by PFGE and WGS was conducted on 24 and 6/24 isolates, respectively. Results: 24/50 CRAB (n=2 from Varzi and Stradella, each, and n=20 from Voghera) resulted OXA-23-producers and further studied. Most of the isolates were from surveillance rectal swabs (n=8; 33,3%), followed by low respiratory tract (n=6; 25%), urine (n=4; 16,7%), blood and wound (n=3; 12,5% each) samples. 11/20 OXA-23-AB from Voghera and all isolates from Varzi and Stradella were collected in the Medicine wards. The remaining nine isolates from Voghera were collected in four different wards. All isolates showed resistance to ciprofloxacin, gentamicin, imipenem, cefepime, cefoxitin, and ceftazidime. More than 95% were resistant to amikacin, meropenem and levofloxacin; and more than 85% to tobramycin and trimethoprim/sulfamethoxazole. All the strains were confirmed OXA-23 positive; 2/12 presented *sul1* gene, 5/12 the *sul2* variant, 3/12 *sul1* and *sul2*. *aac(6')-Ib* gene was identified in 5/12 isolates, *armA* in 11/12 isolates, with co-occurrence of *aac(6')-Ib* in 5/11 cases. Six distinct pulsotypes were identified: the outbreak clone A with eight subtypes (A1-A8; 16/24 isolates), in Voghera only; clone B-B1 (3/24 isolates); clone C-C1 (2/24 isolates). 3/24 were singletons (D-F). Six strains were subjected to WGS showing the circulation of the ST195, ST208, ST218, and ST369 (Bartual scheme); ST2 (Pasteur

scheme). ST195 was found only in Stradella, ST218 in Varzi and Voghera, while ST369 in Stradella and Voghera hospitals. cgMLST scheme and SNPs analyses showed a 1,331 SNPs difference between PFGE clones C-Varzi and E-Voghera (ST218); and 1,845 SNPs between PFGE clones B-Stradella and F-Voghera (ST369). Discussion and conclusions: The circulation of multiple OXA-23-AB lineages was documented within the ASST of Pavia. Clone A-ST208 caused an outbreak in the Medicine ward during February-March 2023, persisting until August 2023. WGS results integrated with epidemiological data, enabled a detailed reconstruction of the transmission dynamics and evolutionary trajectory of this clinically significant pathogen.

189 - Phylogenetic mixing and undetected circulation of multiple C. auris lineages following recognition of imported cases in Italy

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Phylogenetic mixing and undetected circulation of multiple *C. auris* lineages following recognition of imported cases in Italy

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Introduction

Candida auris is a multidrug resistant pathogenic yeast causing nosocomial outbreaks globally, ranking among most critical fungal pathogens according to WHO (1). Its global dissemination has been driven by emergence of genetically unrelated clades (I-VI), whose typing by whole-genome sequencing (WGS) proved useful to track the spread of *C.auirs* in healthcare settings (2). Here we present a genomic snapshot of *C.auris* lineages circulating in Italy, providing evidence of increased clonal heterogeneity following multiple introductions from epidemiologically unrelated geographical areas.

Material and Methods

Publicly available genomes of *C.auris* isolated in Italy since 2009 were retrieved from NCBI databases. WGS of a *C. auris* isolate from a patient admitted to IRCCS Ospedale Policlinico

San Martino (HSM), Genoa (Italy), following a prolonged ICU stay in Tirana (Albania) was performed as previously described (3). Sequence data of international isolates (n=110) from validated outbreak benchmark datasets was also included (4). Phylogenetic analysis was carried out by core-genome single-nucleotide polymorphisms (cgSNPs) using Snippy. Maximum-likelihood phylogenetic trees were constructed on concatenated cgSNPs using IQ-TREE. The status of genes involved in antifungal drug resistance was assessed by BLAST.

Results

Overall, 174 *C. auris* genomes were analyzed. Isolates from Italy were from tertiary-care facilities located in Genoa (HSM, n=61), Milan (n=1) and Bari (n=2). Phylogenetic analysis resolved all isolates within the Sout Asian clade I. All outbreak isolates from HSM clustered within subclade Ic (mean cgSNPS: 9±5), along with outbreak isolates from the U.S (Figure 1). The putative imported case from HSM and the two cases from Bari clustered together within subclade Ib (mean cgSNPS: 5±4), being all epidemiologically linked to Albania. The isolate form Milan was also resolved in subclade Ib, being epidemiologically linked to Greece. Alterations in key genes associated with antifungal resistance were also detected (Figure 1).

Conclusion

Spread of *C.auris* in Italy is currently associated with phylogenetic mixing of diverse clade I lineages (Ib, Ic) following detection of imported cases. Genomic data suggests undetected circulation of clade Ib *C.auris* isolates in Albania, where no cases were to date notified to our knowledge.

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Figure 1

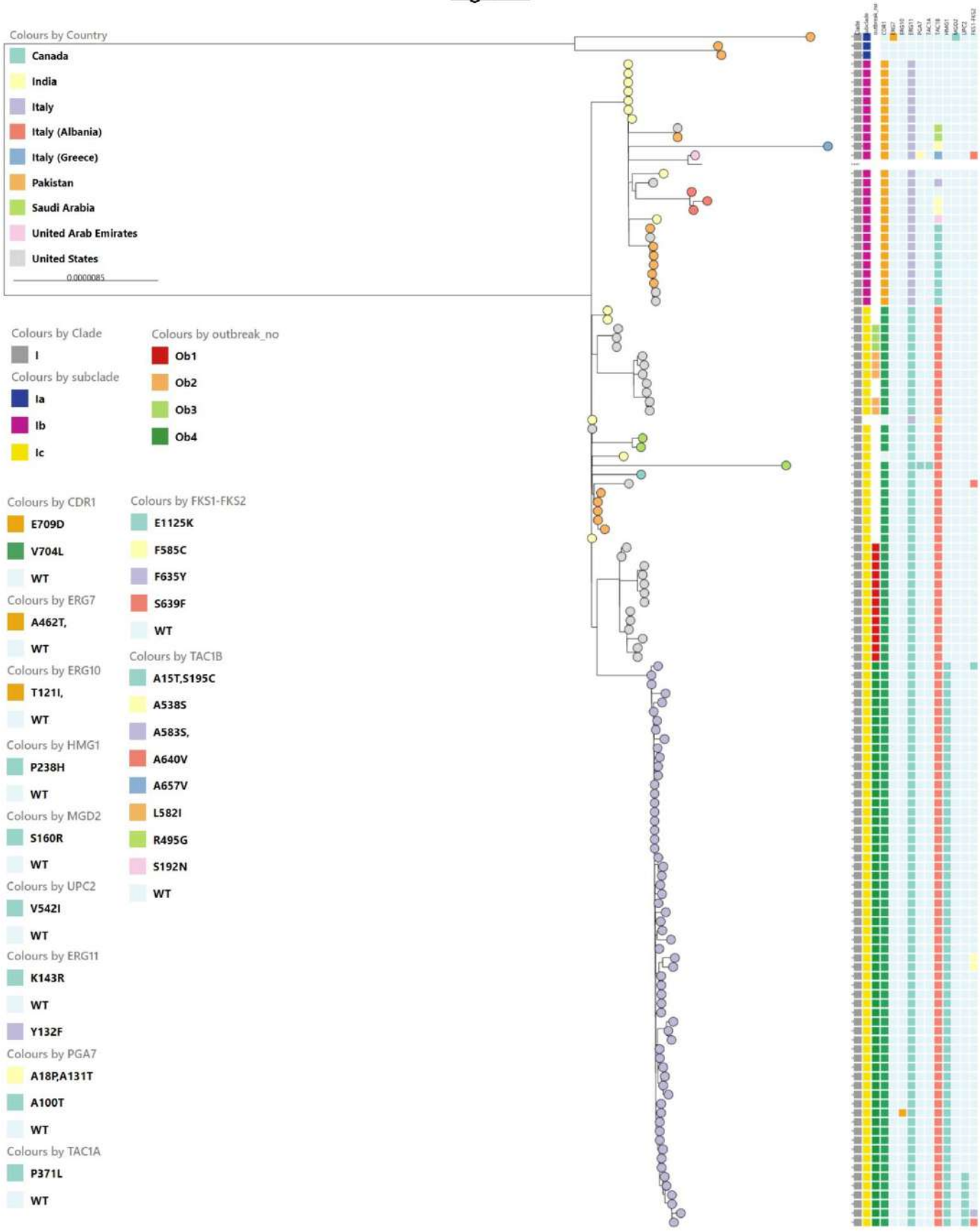

Figure 1. Maximum likelihood phylogeny of *C. auris* isolates from Italy (n = 64) and from benchmark datasets including cases (n=64) from clade I (South Asian). The colours of the isolate tips represent the country of isolation. Details about subclade designation and genetic mutations associated with azoles (*CDR1, ERG11, TAC1B, HMG, UPC, ERG7, ERG10, PGA7, TAC1A, MGD2*) and echinocandins (*FKS1*) resistance are shown.

190 - Investigating Whole Genome Diversity of Antimicrobial Resistant (AMR) Bacteria and Identification of AMR-Associated Genomic Regions

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Investigating Whole Genome Diversity of Antimicrobial Resistant (AMR) Bacteria and Identification of AMR-Associated Genomic Regions

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Introduction: Antimicrobial resistance (AMR) is a significant global health challenge. The comprehensive exploration of whole genomes and identification of potential new AMR-associated genetic elements remain critical areas of research. This research aims to identify with Machine Learning (ML) techniques novel genetic markers that are causative or associated with resistance, providing deeper insights into the genetic mechanisms underlying resistance phenotypes. This analysis has the potential to reveal key drivers of microbial evolution and inform the development of more effective surveillance and treatment strategies. **Materials and Methods**: The first phase of this research focused on N. gonorrhoeae isolates, downloaded as FASTA files from the public databases. Following the download, comprehensive quality control was undertaken to evaluate completeness, contamination levels, GC content, and overall integrity of each genome assembly. The resulting dataset was then subjected to taxonomic verification, which confirmed that all the genomes were correctly assigned to N. gonorrhoeae, with no misclassified entries. Following quality control and classification, the genome sequences were annotated. **Results**: During the first phase of this study, a total of 22,504 N. gonorrhoeae genomes were downloaded. Genomes were retained if they met the following quality criteria: a maximum contamination level of 5% or less, a genome size ranging between 2.0 and 2.3 Mb, a GC content between 50% and 54%, and a coding sequence (CDS) count

between 2,000 and 2,700. The metadata linked to each genome enabled the analysis of resistance trends across our N. gonorrhoeae dataset. The comparison of resistance phenotypes between sexes revealed the prevalence of cephalosporin susceptibility, with around 7000 male and 1000 female isolates susceptible, whereas less than 50 isolates of either sex were resistant. By contast, the temporal analysis of antibiotic resistance revealed a consistently high level of cephalosporins susceptibility over the years, with resistance generally staying low. **Discussion and Conclusion**: The next phases of this project will implement ML pipelines to predict AMR phenotypes from genomic data. Supervised ML pipelines can be trained on paired genome-phenotype datasets using algorithms such as logistic regression, random forests, and gradient-boosted trees. This ML-based approach has the potential to reveal novel genetic patterns and regions that contribute to phenotypic resistance profiles, improving our understanding of the genetic basis of resistance. Tracking their distribution across temporal scales, these elements may also serve as evolutionary markers, offering insights into the genomic adaptations bacteria employ in response to antibiotic exposure.

193 - Genomic analysis of carbapenem-resistant Pseudomonas aeruginosa isolates: spread of ST235 and ST309 high-risk clones.

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Genomic analysis of carbapenem-resistant *Pseudomonas aeruginosa* isolates: spread of ST235 and ST309 high-risk clones.

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Introduction**:** Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) has become a high priority pathogen according to WHO. *Pseudomonas* strains exhibit various environmental adaptability due to the larger genome encoding several virulence and resistance factors. Herein, Whole Genome Sequencing (WGS) was performed to provide insights into outbreak surveillance, drug-resistance monitoring, and circulation of virulence mechanisms. Materials and Methods**:** Between January 2020 and June 2024, ten clinical CRPA isolates were collected. Antibiotic susceptibility testing was performed by broth microdilution and Vitek2 (bioMérieux) methods. Genomic DNA was extracted using QIAAMP DNA MINI KIT (Qiagen). The Illumina paired-end libraries were constructed using a DNA prep Kit (Illumina), according to the manufacturer's guidelines. WGS was performed by the MiSeq platform. Antibiotic resistance genes were detected using Comprehensive Antibiotic Resistance Database (CARD) and Resfinder. Multi-locus Sequence Typing (MLST) prediction was performed using MLST database. Virulence genes were determined by the Virulence Factor Database and pseudomonas.com. Results**:** *P. aeruginosa* strains were isolated from clinical specimens including blood culture (n= 1), urine (n= 3), bronchial aspirate (n= 2), bronchoalveolar lavage (n= 1), throat swab (n= 2), and wound swab (n= 1), then seven sequence types (STs) were identified: ST760, ST309, ST3236, ST3505, ST621, ST207, and ST235. The most relevant virulome genomic regions, such as *alg44, alg8, algK, algX* associated with biofilm formation and *cupA12345, cupB123456* related to fimbriae type I genes were identified in all analyzed isolates. Additionally, *exo*U type III secretory system-related gene was detected in 7/10 strains, while genes involved in pyoverdine biosynthesis (*pvdA, pvdD, pvdE, pvdF, pvdI, pvdJ, pvdL, pvdM, pvdN*) were revealed in 10/10 strains. Moreover, the resistance *aac*(3')-Ic, *aac*(6')-Ib,

*aac(*6')-Ic, *aph*(3')-IIb, catB7, *bla*GES-7, *bla*OXA-50 and *bla*OXA-488, *bla*VIM-1, *sul*1, *bla*PAO, *fos*A1 genes were found. Discussion and Conclusions**:** Two high-risk clones ST235 and ST309 were identified. In particular, ST235 determine a much higher mortality that other high-risk clones. Four out of ten strains displayed the co-resistance to ceftazidime–avibactam and ceftolozane–tazobactam related to presence of *bla*OXA-488, interestingly strain#3, carrying this gene, was sensitive to both antibiotics. Our study provide novel insights into circulation of the strains. Furthermore, the results contributed to understanding the antimicrobial resistance pattern existing among *P. aeruginosa* isolates. Molecular tools are necessary to prevent and control the spread of resistant strains in hospital settings.

210 - Toward the Genomic Characterization of the Enigmatic 'Candidatus Saccharimonadia' through Targeted Enrichment from Human Saliva

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Toward the Genomic Characterization of the Enigmatic '*Candidatus* Saccharimonadia' through Targeted Enrichment from Human Saliva

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Introduction

"*Candidatus* Saccharimonadia" is an important but poorly characterized bacterial lineage, generally present in human oral microbiota, with a frequency of ~5%. Recent *in vivo* evidence revealed, the bacterium can have immuno-modulating effects and it has been found enriched in patients with inflammatory conditions, such as periodontitis, inflammatory bowel disease and food allergy. "*Ca*. Saccharimonadia" exhibit small cell-sized, reduced genomes and an epibiotic lifestyle, which makes them difficult to culture with standard techniques. Their fastidious nature and low abundance poses major challenges for study, as current genomic approaches based on co-cultivation or shotgun metagenomics, are limited by cost and complexity. A reliable and accessible protocol to enable the genomic characterization of this group is needed.

Materials and Methods

We developed a simple, cost-effective protocol (SE: Saccharimonadia Enrichment) to enrich "*Ca*. Saccharimonadia" directly from human saliva. The SE protocol consists of two mandatory steps: i) detachment of bacteria from their host cells; ii) size-based separation using a

centrifugation gradient. Optionally, free DNA can be removed by using the Propidium Monoazide, before final DNA extraction. This method was applied to fresh human saliva samples, and 16S and shotgun metagenomics were used to assess the enrichment and to obtain "*Ca*. Saccharimonadia" genome assemblies.

Results

The SE protocol enriched "*Ca*. Saccharimonadia" in the saliva microbiota from ~5% to ~80% and, after the enrichment, less than 10% reads were from humans. Thus high quality genome assemblies were obtained, with statistics comparable or better than "*Ca*. Saccharibacteria" genomes available in public databases. It was possible because the enrichment procedure reduced the background signal from non-target organisms, thereby increasing the accuracy of the sequencing results.

Discussion and Conclusions

The SE protocol can represent an advancement in studying "*Ca*. Saccharimonadia" offering a direct genomic access to ultra-fastidious members of the human microbiota. By enabling efficient genome sequencing, it opens new perspectives for exploring the role of these bacteria in the oral microbiota and their involvement in inflammatory and immune-mediated diseases as well as microbial-host interactions. The SE protocol offers researchers a practical tool to access previously hidden members of the human oral microbiota that today remain an underexplored, immunologically active niche potentially involved in early immune priming. Moreover, this methodology requires only standard laboratory equipment commonly available in microbiology labs and significantly lowers the sequencing depth, which further reduces the computational resources.

251 - Human Papillomavirus type 56 (HPV56) detection in women with cervical dysplasia: prevalence and lineages distribution in two different Italian Regions

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Human Papillomavirus type 56 (HPV56) detection in women with cervical dysplasia: prevalence and lineages distribution in two different Italian Regions

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Introduction: Persisting infection of high-risk Human Papillomavirus (hrHPV) is the main cause of cervical cancer. More than 200 HPV types are known but only 12 (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) are recognized as carcinogenic for humans by the International Agency for Research on Cancer (IARC). HPV16 and HPV18 are the most common types associated with cancer and consolidated data are reported for these two hrHPV types. Less is known for other hrHPVs but the introduction of extended genotyping has also allowed to better understand the distribution and association with disease of less common genotypes. The objective of this study was to investigate the prevalence of HPV56 and its specific lineages and sub-lineages among women referred to colposcopy for a recent cervical dysplasia.
Materials and Methods: Cervical samples were presently collected from 363 women referred to colposcopy at IRCCS San Gerardo dei Tintori Hospital (Monza, Lombardy) and from 81 women referred to Coordinamento Consultori Familiari, ASSL Sassari (Sardinia). HPV testing and genotyping was performed using Anyplex™II HPV (Seegene). HPV56 positive samples were further investigated using previously described PCR protocols to obtain E7 oncogene sequences and analysis was performed using MEGA software.
Results: HPV56 was identified in 27/363 (7.4%) women in Lombardy and in 3/81 (3.7%) of those enrolled in Sardinia, respectively. 73.3% (22/30) were detected as multiple hrHPV infection with

other types. Presently, 19 E7 sequences have been analysed and the phylogenetic tree is reported in Figure 1. Ten sequences belonged to lineage A (7 to sub-lineages A1 and 3 to sub-lineages A2) and 9 to lineage B.

Discussion and Conclusions: Although HPV56 is associated with less than 1% of cervical cancer, it is important to continue to investigate the role of this genotype, not included into vaccines coverage. These preliminary results implement epidemiological data regarding HPV56 infection, but a larger number of patients with infection and HPV56 sequences are required to better investigate the role and association of this genotype with cervical cancer and/or preneoplastic lesions.

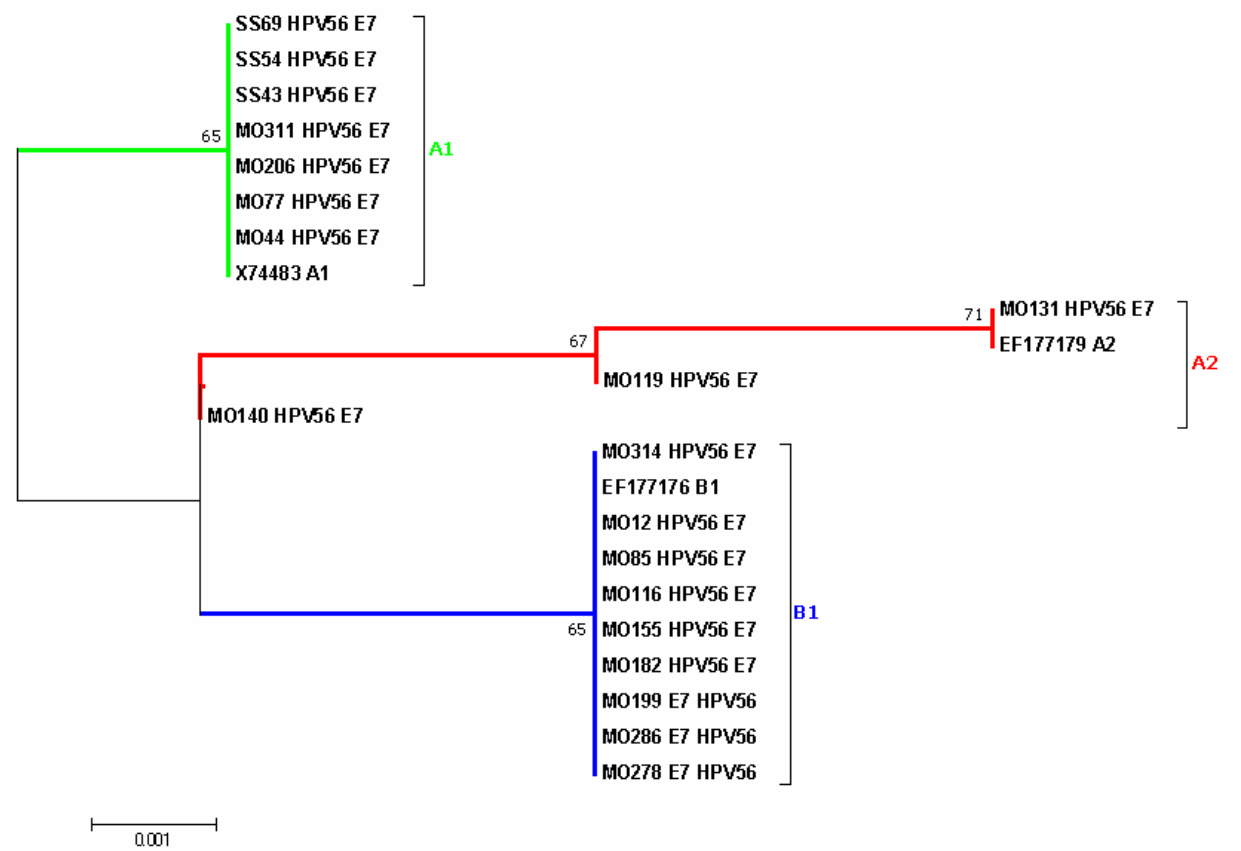

Figure 1: HPV56 E7 phylogenetic tree

287 - Prevalence and Molecular Characterization of β-Lactamase and Quinolone Resistance Genes in Uropathogenic Escherichia coli (UPEC) Isolates

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Prevalence and Molecular Characterization of β-Lactamase and Quinolone Resistance Genes in *Uropathogenic Escherichia coli* (UPEC) Isolates

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1. **Introduction**

   Uropathogenic E. coli (UPEC) is the main cause of urinary tract infections (UTIs) in community and healthcare settings. The emergence of ESBL - and carbapenemase-producing strains, along with quinolone resistance, complicates treatment. Detecting resistance genes is key to tackling multidrug resistance and guiding control strategies.
2. **Materials and Methods**

A total of 91 UPEC isolates from Algerian patients exhibiting intermediate or resistant phenotypes to β-lactams or quinolones (Kirby-Bauer disk diffusion) were analyzed for antimicrobial resistance genes (ARGs). β-lactamase genes were detected using Quadruplex PCR (SET1: *blaTEM, blaSHV, blaCTX-M IV, blaOXA*; SET2: *blaCTX-M I/II, blaCMY II, blaDHA*). Additionally, the presence of ARGs conferring resistance to quinolones (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) was assessed.

3. **Results**
4. ARG screening revealed β-lactamase genes in 95% (87/91) and quinolone resistance genes in 25.3% (12/46) of UPEC isolates. The predominant genes were *blaTEM* (63.7%) and *qnrB* (39.1%). The *blaTEM/blaCTX-M I* combination was the most common (P = 0.024). Significant correlations were found between *blaTEM* and resistance to amoxicillin, tetracycline, and ticarcillin/clavulanic acid; *blaOXA* and piperacillin/tazobactam*; blaCTX-M II* and amoxicillin/clavulanic acid and gentamicin. Co-occurrence of *blaOXA/qnrB, blaSHV/qnrS,* and *blaDHA/qnrD* ($P<0.05$) suggests linkage on mobile genetic elements such as transposons, integrons or plasmids.
5. **Discussion and conclusion**

This study revealed a high prevalence of β-lactamase (95%) and quinolone resistance genes (25.3%) in UPEC isolates, with *blaTEM* and *qnrB* as the predominant genes. Emphasising molecular detection and characterisation of resistance determinants, the research provides essential data to guide therapeutic decisions and strengthen antimicrobial stewardship. Significant gene-antibiotic correlations and co-occurrence patterns suggest possible genetic linkage via mobile elements. These findings emphasise the need for molecular surveillance to guide treatment, contain resistant strains, and preserve antibiotic efficacy in both hospital and community settings.

355 - Preliminary Evaluation of Mobilome and Virulence Gene Content Impact in Staphylococcus aureus Sequence Type 8 Isolates During In Vitro Cellular Internalization

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Preliminary Evaluation of Mobilome and Virulence Gene Content Impact in Staphylococcus aureus Sequence Type 8 Isolates During In Vitro Cellular Internalization

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 8 (ST8), also known as ST8-MRSA-IV or "USA300", is globally distributed and associated with significant virulence. About 15% of the *S. aureus* genome comprises the mobilome—mobile genetic elements such as Staphylococcal Pathogenicity Islands (SaPIs), SCC elements, prophages, plasmids and transposons, which play a crucial role in genome evolution and acquisition of stress-response and virulence factors. In a previous study, we evaluated the internalization capacity of five clinical ST8-MRSA-IV strains in human MG-63 osteoblasts. Here, we focus on two of these clinical isolates (both ST8-MRSA-IV and PVL-positive) to investigate differences in internalization in relation to their virulence gene profiles and mobilome content.

Materials and Methods

Two clinical isolates, CT-1 and CT-2, along with the reference strain *S. aureus* ATCC 12598, were used to infect MG-63 osteoblasts at an MOI of 100:1. Extracellular bacteria were removed with

lysostaphin treatment. Intracellular bacteria were quantified at 3 to 24 hours post-infection (hpi) by blood agar plating. Internalization was also assessed by imaging flow cytometry (IFC) using vancomycin-BODIPY staining at 24 hpi. Whole-genome sequencing (WGS) was performed using Nanopore and Illumina platforms. Virulence genes, resistance genes (resistome) and mobile genetic elements (mobilome) were analyzed bioinformatically.

Results

Both clinical strains could internalize into osteoblasts but showed markedly different efficiencies: CT-1 had 13.3% internalization, while CT-2 showed 51.4%, comparable to the control strain ATCC 12598 (51%). The resistome analysis revealed that CT-1 carried *blaZ* and *mecA*, while CT-2 harbored a broader resistance profile: *cat*, *erm(A)*, *tetK*, *ant(9)-Ia*, and *mecA*. The *pvl* gene was present in both strains' core genome.

Genomic comparison showed key differences in virulence genes:

- CT-1 had a 1.6 kb deletion in the *sraP* gene and carried *sdrC*, *sdrD* and *sdrE*.
- CT-2 carried a complete *sraP* and the adhesin genes *sdrC*, *esaD* and *clfA*, but lacked *sdrD*.

Mobilome analysis indicated:

- CT-1 had a larger mobilome (6.12% of genome), including the plasmid pSK67 (*blaZ*), a novel transposon and four novel prophages.
- CT-2 had a smaller mobilome (4.66%), including chromosomally integrated pUB110, another plasmid with aminoglycoside resistance genes and two novel prophages.

Discussion and Conclusions

Despite their shared clonal background, CT-1 and CT-2 differ significantly in their internalization into osteoblasts. This may be due to differences in virulence gene content, especially in the *sraP* gene. *SraP* is a serine-rich, glycosylated adhesin involved in adhesion, invasion and immune evasion. Its partial deletion in CT-1 may explain reduced internalization. Conversely, CT-2's full-length *sraP* and presence of *ClfA* might account for its higher internalization efficiency. While CT-2 lacks *sdrD*, this gene is associated more with chronic infection adaptation rather than acute invasion.

These preliminary findings suggest a potential role for specific mobilome elements and virulence genes in modulating internalization and pathogenicity. Further studies are needed to validate these observations and explore gene expression and protein function related to internalization and persistence.

357 - Genomic Profiling of Enterococcus faecalis Extracellular Vesicles from Vancomycin-sensitive and resistant Strains

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Genomic Profiling of *Enterococcus faecalis* Extracellular Vesicles from Vancomycin-sensitive and resistant Strains

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Introduction

The functional role of bacterial extracellular vesicles (EVs), nanosized membrane structures released by Gram-positive and Gram-negative bacteria, has attracted growing scientific attention, and our work focuses on exploring this aspect. *Enterococcus faecalis*, a Gram-positive opportunistic pathogen, is frequently isolated in nosocomial infections and shows remarkable adaptability to antibiotics, including vancomycin. We isolated and genomically characterised EVs released by a vancomycin-sensitive *E. faecalis* strain (ATCC 29212) and a vancomycin-resistant one (ATCC 51299), comparing the genetic cargo of the vescicles with that of the corresponding parental genomes.

Materials and Methods

Culture supernatants from *E. faecalis* strains ATCC 29212 and ATCC 51299 were collected and filtered through 0.22 µm membranes. Extracellular vesicles (EVs) were isolated by size exclusion chromatography (SEC) and characterized using nanoparticle tracking analysis (NTA)

with the NanoSight system. DNA was extracted from EV pellets using the Qiagen DNeasy Blood and Tissue Kit and quantified using a Qubit fluorometer. Sequencing was performed on the Illumina MiSeq platform, and bioinformatic analysis was conducted to identify virulence factors and antimicrobial resistance genes.

Results

EVs derived from the vancomycin-sensitive strain ATCC 29212 carried capsule regulator *cpsC*, cytolysin modulators *cylR1* and *cylR2*, sortases *srtC* and *srtA*, and pheromone genes *cad*/*cCF10*. Additionally, the arsenic-resistance genes *arsA*, *arsD* and *arsR* were detected. In contrast, EVs from the vancomycin-resistant strain ATCC 51299 lacked identifiable virulence genes but harbored key resistance determinants, including *ermB* and the complete *vanB* cluster, conferring high-level glycopeptide resistance. Parental strains exhibited a broader spectrum of both virulence and resistance genes, highlighting selective packaging of EV cargo.

Discussion and Conclusions

EVs from the two *E. faecalis* strains displayed markedly different genetic content, possibly reflecting distinct roles in pathogenesis and resistance dissemination. The enrichment of virulence-related genes in EVs from the vancomycin-sensitive strain, may reinforce host-pathogen interactions and improve environmental persistence, thereby favouring early colonisation. In contrast, the exclusive presence of *vanB* and *ermB* in resistant strain-derived EVs underscores the vesicles potential in spreading resistance elements. The absence of virulence genes in the VRE-derived EVs raises intriguing questions about possible trade-offs between resistance acquisition and pathogenic potential. These findings provide novel insights into the molecular cargo of *E. faecalis* EVs and their potential role in antimicrobial resistance dissemination.

360 - Characterization of pediatric oral microbiota by next-generation sequencing (NGS) of saliva samples and supragingival plaque: a new tool to assess effectiveness of gingivitis treatment

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Characterization of pediatric oral microbiota by next-generation sequencing (NGS) of saliva samples and supragingival plaque: a new tool to assess effectiveness of gingivitis treatment

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Introduction: The oral cavity has specific niches where different bacterial taxa play a significant role in ecological relationships. Bidirectional interactions with the host have an effect on the oral microbiota, shaping composition, distribution patterns and community behaviors of the oral communities. Changes to the oral microbiome can cause disruption of its symbiosis relationship with the host, which can result in both oral and systemic diseases. Gingivitis, usually caused by an overgrowth of potentially pathobiont bacteria in the oral cavity, is most common during puberty. In order to prevent oral diseases, novel therapeutic approaches to restore a healthy oral microbiota in children aged between 7-18 have been considered.

Material and Methods: Oral microbiome was evaluated at baseline (T0), after 10 days (T1) and after 30 days (T2) since mouthwash treatment, by a metataxonomy approach based on next-generation sequencing (NGS), to analyse saliva samples and supragingival plaques from ten patients.

Results: NGS based results showed differences between plaque and saliva microbiota profiles due to treatment over time. In particular, it was observed an increase of Streptococcus,

Granulicatella and Lautropia and a decrease of Prevotella, Veillonella and.Campylobacter in both microbiota. At T2, slight regressions occurred, partially restoring the initial conditions.

Discussion and Conclusion: The oral microbiota analysis showed the role of specific bacteria in the development of oral diseases, with the increase of potential biomarkers of restored oral healthy microflora and the reduction of oral pathobiont indicators. Despite the regression at T2, the microbiome remained within healthy biomarker ranges. Based on the clinical outcomes, additional microbiological analysis will be carried out to examine microbial changes.

361 - The study of the age-related composition of the gut mycobiome and microbiome in patients with ulcerative colitis.

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1. Introduction

The delicate balance of the gut can be upset by changes to the microbiome and mycobiome that occur with age. This can lead to increased inflammation, impaired gut barrier function and altered immune responses. These changes can contribute to the development and progression of inflammatory bowel diseases (IBDs), such as ulcerative colitis (UC) and Crohn's disease, by exacerbating the inflammatory response.

2. Materials and methods

We retrieved 73 bacterial and 69 fungal gut metagenomic profiles from pediatric and adult UC patients from the NCBI database. Ecological analyses were performed using alpha- and beta-diversity. To evaluate differences in microbiota and mycobiota profiles between pediatric and adult UC groups, we applied unsupervised and supervised multivariate analyses and a univariate analysis.

3. Results

The UC cohort comprised 26 pediatric and 47 adult patients. Of a total of 1,075 fungal and 8,154 bacterial amplicon sequence variants (ASVs), we obtained 149 and 353 genera, respectively. Alpha diversity analysis revealed a statistically significant increase in fungal richness in adults compared to children. The beta diversity analysis revealed distinct clusters of samples belonging to the pediatric and adult UC cohorts.

> Comparative analysis of fungal ASVs revealed specific biomarkers associated with the pediatric group, including Saccharomycetes, *Aureobasidium*, and *Cladosporium*, whereas the adult group showed biomarkers including *Wickerhamomyces*, *Betamyces*, *Fusicolla*, *Aphelidium*, *Cutaneotrichosporon*, *Vishniacozyma*, Chytridiomycota, and *Clavispora*.

We then assessed the gut microbiota profiles to identify differences in bacterial composition between pediatric and adult UC patients. Our results revealed that there were no statistically significant differences in either alpha- or beta-diversity indices between the two groups. Furthermore, comparing the bacterial ASVs between the pediatric and adult groups did not reveal a statistical difference.

4. Discussion and Conclusions

In UC, the gut mycobiota profile is influenced more by age than the gut microbiota profile. Identifying age-specific fungal markers could inform future diagnostic and prognostic evaluations, paving the way for personalised strategies and therapies for patients of different ages.

07 7. Il futuro delle reti e della ricerca

50 - Experimental design to test the antibacterial action of ZnO microcrystals within the scope of the project SOTERIA

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Experimental design to test the antibacterial action of ZnO microcrystals within the scope of the project SOTERIA

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Introduction: The interdisciplinary SOTERIA (Smart biopolymeric ZnO Nanowires composites for enhanced antibacterial activity) project aims to develop a novel class of highly efficient antibacterial systems based on ZnO, at the chemistry-biology interface. Given the simple tunability of ZnO morphology through synthesis condition manipulation, we will engineer multiscale structures. Variations in these structures are anticipated to influence the material stability and dispersibility in aqueous environments impacting its antibacterial performance. The activity against bacteria is due to the capabilities of generating Reactive Oxygen Species (ROS) inducing DNA and protein damage and zinc ion release, modifying the homeostasis of the aqueous environment. Different types of ZnO microcrystalline powder were synthesized. Their antibacterial action was tested against two reference strains exhibiting different structural/physiological features (*Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 29213). This study elucidates how the concentration and morphology of microcrystals obtained could impact the ZnO activity against bacteria.

Materials and Methods: The synthesized ZnO microcrystals (ZnO MC) form a colloid in an aqueous solution that precipitates rapidly. Broth micro- and macro-dilution methods for the evaluation of the antibacterial activity, were performed in Cation Adjusted Mueller-Hinton Broth plus 3% acetic acid V/V as ZnO solvent, to determine the MIC value. Analyses were performed to quantify the ROS and zinc ions production and justify the results obtained from the broth tests. ZnO MC antibacterial activity without solvent, was evaluated by agar dilution method that allowed powder uniform dispersion in the culture medium.
Results: Preliminary results show that the use of ZnO MC in broth or agar has different antibacterial action depending on the powder characteristics and its dispersibility in the aqueous environment. MIC values ranging from 512 to 128 mg/L showed no significant antibacterial differences against Gram-positive and Gram-negative models, using either dark or light conditions.
Discussion and Conclusions: The antibacterial activity of ZnO MC was verified for the two reference strains both in aqueous environment and solid culture medium. Tests with stabilizing agent and mechanical support are planned to overcome problems related to the poor dispersibility of ZnO MC in aqueous systems. The interdisciplinarity of the project is allowing to produce and test cellulose supports functionalized with APTES ((3-Aminopropyl) triethoxysilane) molecules for prolonged release medical applications.
Acknowledgements: This study was supported by PRIN 2022 (Soteria), PNRR M4.C2.1.1. 2022WZK874. CUP: H53D23004700006.

66 - Preliminary data of metagenomic profiling of the circulating microbiome in cardiovascular diseases

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Preliminary data of metagenomic profiling of the circulating microbiome in cardiovascular diseases

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INTRODUCTION

Heart diseases continue to be a top reason for sickness and death worldwide, but we still don't know much about the role of microbial circulating DNA in our bloodstream. Emerging evidence hint that microbes moving into the bloodstream and systemic inflammation might contribute to the worsening of cardiovascular diseases (CVD), but we need a full picture of the microbes in heart disease patients' blood. This study sets out to look at the link between high levels of free-floating DNA, microbial DNA, and heart diseases, with the aim to find possible microbe markers and therapeutic targets.

MATERIALS AND METHODS

The study currently included 40 patients diagnosed with different types of heart diseases (including valvular abnormalities, impaired blood flow, congenital heart defects, and hypertensive heart issues) and 10 healthy controls. Our team measured free-floating DNA in blood plasma using a PicoGreen test, while bacterial (16S rRNA) and human (beta-globin) DNA levels with qPCR. We used shotgun sequencing to map out microbial and viral communities. Statistical analysis was performed with Kruskal-Wallis tests and receiver operating characteristics (ROC) curve analysis.

RESULTS

Heart disease patients had up to 52 times more free-floating DNA (2100.78 ng/mL) than healthy people (40.25 ng/mL; $p < 0.001$). Bacterial DNA load showed a big increase (5500.23 vs. 430.90 copies/mL; $p < 0.001$), and patients with congenital heart disease (CHD) had the highest levels (34000.78 copies/mL). CVD patients had a much higher bacterial-to-human DNA ratio (30.12

vs. 5.25; $p < 0.001$). When we looked at the DNA makeup, we found that Actinomycetota (47%) and bacteriophages (65%) were the main types in CVD patients, while Pseudomonadota (48%), and eukaryotic viruses (77%) were predominant in controls. Furthermore, CVD patients also exhibited higher levels of inflammation markers, including CRP (Bio-Rad, CA, USA, and Thermo Fisher Scientific, Waltham, MA, USA) and IL-6 (BD OptEIA™ Human IL-6 ELISA Set, BD, Franklin Lakes, New Jersey, USA) and dyslipidemic profiles ($p < 0.001$).

DISCUSSION AND CONCLUSIONS

This research shows that circulating microbial DNA and an imbalanced microbiome play a big part in how CVD develops. The significant elevation of bacterial DNA relative to human DNA, and the predominance of specific microbial taxa in CVD patients, suggests that microbial translocation and dysbiosis may actively contribute to disease pathogenesis through the promotion of systemic inflammation. The high levels of circulating DNA and microbial markers could be useful for diagnosis, and they give us a starting point to create new treatments that target the microbiome to slow down CVD.

271 - Role of Microbiome in Colon Cancer Among Italian Population: A Next-Generation Sequence-Based Study

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Role of Microbiome in Colon Cancer Among Italian Population : A Next-Generation Sequence-Based Study

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Introduction: Colon cancer (CRC) is one of the main leading causes of cancer related deaths all over the world and its incidence is steadily increasing in Italy. New research indicates that the gut microbiota has a significant influence on the pathogenesis of colon cancer. Modifications in the makeup of microorganisms may have an impact on carcinogenesis, immunological response, and inflammation. The gut microbiota of Italian CRC patients is profiled in this study using next-generation sequencing (NGS). Our goal is to find possible

microbial relation by comparing the microbial patterns in CRC patients and healthy controls. Our research could help develop more individualized treatment plans and better risk assessments.

Materials and Methods: A retrospective study was performed on a cohort of 16 patients (8 with colon cancer and 8 healthy controls). Colon tissues were collected and processed as formalin-fixed paraffin-embedded (FFPE) samples. DNA was extracted from FFPE specimens, and the microbiome was analyzed through amplification of the 16S rRNA gene targeting the V3-V4 regions. Next Generation Sequencing were assessed for the comparison of genomic / transcriptomic differences between two conditions or groups. Pairwise comparison of microbial species between colon cancer (CRC) and control groups identified several taxa with significantly different relative abundances (Padj < 0.05).

Results: Our results showed that species depleted in CRC included *Coprococcus comes*, *Eubacterium ramulus*, *Dorea formicigenerans*, *Eubacterium hallii*, *Eubacterium rectale,* and *Roseburia intestinalis*, with fold reductions (FR) ranging from approximately 3-8 times lower in CRC. These taxa are known producers of short-chain fatty acids, suggesting a potential loss of beneficial functions in CRC. Conversely, some species such as *Lactococcus lactis, Solobacterium moorei*, and *Fusobacterium nucleatum* showed elevated levels in CRC, though their overall abundance remained low. Notably, *Roseburia intestinalis* displayed an unusually high fold ratio (FR = 162.6), possibly due to inter-individual variation or sample-specific enrichment.

Discussion and Conclusions: This study investigates differences in gut microbiota composition between colon cancer (CRC) patients and healthy controls. Using mean abundance data and statistical tests, we assessed microbial species-level changes. Although some species showed numerical differences in mean abundance, adjusted p-values indicate no statistically significant differences between groups after multiple testing correction. These findings suggest subtle or non-significant shifts in gut microbial profiles in CRC within the analyzed cohort.

294 - The effect of a commercial probiotic preparation on immunosuppressant drugs and on their toxicity in an in vitro model of the intestinal barrier

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The effect of a commercial probiotic preparation on immunosuppressant drugs and on their toxicity in an *in vitro* model of the intestinal barrier

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Introduction: Intestinal dysbiosis often occurs in recipients of solid organ transplants and increases the risk of graft rejection. This effect partly depends on the ability of the dysbiotic microflora to degrade or sequester the immunosuppressant drugs (IMDs), thereby reducing their efficacy or increasing their toxicity. Probiotics are used in clinics to restore normal gut microbiota composition in patients with intestinal dysbiosis, so it is expected that they could correct alterations in the pharmacological behavior of IMDs induced by dysbiosis. However, it is currently unknown whether these microorganisms can affect IMDs directly. In the present study, therefore, we evaluated whether a probiotic preparation might degrade or accumulate the frequently used IMDs mycophenolate mofetil, mycophenolic acid and their metabolite mycophenolate acid glucuronide, or affect their toxicity in an *in vitro* model of the intestinal barrier.
Materials and Methods: A probiotic preparation containing *Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus, Lactobacillus acidophilus, Lacticaseibacillus casei, Streptococcus thermophilus* and *Bifidobacterium lactis* was cultivated in presence of various IMDs (50 µM) at 37°C for six hours under anaerobic conditions. At times 0 and after 2, 4, and 6 h of incubation, microbial growth was assessed by viable plate counts, while the ability of probiotic bacteria to metabolise and/or accumulate IMDs was assessed by HPLC analysis. To evaluate the effect of probiotic preparation on IMD toxicity, a transwell model was employed, wherein the human intestinal cell line Caco-2 was cultivated on a semipermeable membrane

to simulate the intestinal barrier. Caco-2 cell viability was assessed in control conditions and in the presence of IMDs, with or without probiotic-conditioned medium, using the CyQuant assay. The expression of proteins involved in intestinal epithelial integrity, such as tight junction proteins, was analysed by immunoblotting and qPCR, while P-glycoprotein activity was assessed using rhodamine 123.

Results: IMDs did not exhibit bacteriostatic or bactericidal properties against the probiotic preparation tested. Furthermore, HPLC analysis of the culture medium extracts and cell lysates of probiotic bacteria incubated with IMDs showed no anomalous peaks indicative of drug degradation. Additionally, *in vitro* tests on intestinal cells indicated that co-administering IMDs with the probiotic could mitigate the adverse effects associated with IMDs alone.

Discussion and conclusions: The results of this study, funded by Italian Ministry of Health PNRR-MAD-2022-12376812, showed that the probiotic preparation had minimal impact on the tested IMDs. This supports its potential use in correcting dysbiosis in post-transplant patients.

321 - Presystemic metabolism of immunosuppressant drugs by gut microbiota as a risk factor for kidney transplant rejection: mitigation strategies

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Presystemic metabolism of immunosuppressant drugs by gut microbiota as a risk factor for kidney transplant rejection: mitigation strategies

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Kidney transplantation is a lifesaving procedure for patients with end-stage renal failure. Unfortunately, however, around 7% of recipients of kidneys from deceased donors experience acute graft rejection within one year of surgery. Intestinal dysbiosis, which commonly affects kidney transplant recipients (KTRs), is a significant risk factor for organ rejection following transplantation. This may be partly due to the reduced efficacy of immunosuppressant drugs (IMDs). In fact, these drugs may be degraded or sequestered by dysbiotic microorganisms, reducing their absorption. Furthermore, IMD gastrointestinal toxicity may be enhanced, which decreases patient compliance. Therefore, it is expected that therapeutic interventions that restore the normal composition of the intestinal microbiota will correct the alterations in IMD pharmacology induced by dysbiosis. To investigate this hypothesis, we are conducting a project entitled 'Presystemic metabolism of immunosuppressant drugs by gut microbiota as a risk factor for kidney transplant rejection: mitigation strategies'. This project, funded by the Italian Ministry of Health (PNRR-MAD-2022-12376812), aims to investigate whether short-term

treatment with a probiotic preparation improves the pharmacokinetics and/or tolerability of IMDs in a cohort of KTRs. As there is currently no information on the potential impact of probiotic microorganisms on IMDs, preliminary *in vitro* experiments have been conducted to address this issue. The growth and viability of the microbial cells were assessed, as were drug degradation and intracellular accumulation, at baseline and at scalar times from the beginning of the incubation. The impact of probiotics on IMD-induced gastrointestinal toxicity was evaluated *in vitro* using a transwell model of the intestinal barrier. Specifically, CaCo-2 cells were grown onto the semipermeable membrane of the transwell insert until confluence. Then IMDs (50 mM) were added to the upper compartment of the transwell. Cell viability and markers of epithelial integrity were assessed at baseline and at various time points after the start of drug incubation. At the same time points, epithelial permeability was also assessed. Similar experiments were performed using three different commercial probiotic preparations to select the most suitable one for the clinical study. We will present the results of these preliminary *in vitro* studies, as well as the design of the upcoming clinical study.

328 - Community-acquired respiratory infections in hospitalised children: preliminary microbiological data from the SIS-NET paediatric cohort enrolled at Fondazione IRCCS San Gerardo dei Tintori, Monza.

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TOPICS 2025: Il futuro delle reti e della ricerca

Community-acquired respiratory infections in hospitalised children: preliminary microbiological data from the SIS-NET paediatric cohort enrolled at Fondazione IRCCS San Gerardo dei Tintori, Monza.

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Introduction:
The recently established national network SIS-NET ("**S**evere **I**nfections and **S**epsis clinical **NE**twork for identification of clinical and diagnostic markers, immunological monitoring and ***T****arget and tailored* therapies for adults, children and patients admitted to intensive care units") (Bando a Cascata PNRR ref COC-1-2023-ISS-01 id S4-01.P0001) addresses the need to monitor emerging or re-emerging pathogens in patients of all age groups. This project aims to integrate clinical and microbiological data into a centralized national database, to establish robust epidemiological surveillance and to support timely and effective public health measures. This abstract focuses on the preliminary results of respiratory pathogens identified in paediatric patients enrolled by the SIS-NET participating centres of the University of Milano-Bicocca.

Materials and Methods:

Enrolment of hospitalised children with severe community-acquired respiratory tract infections, as part of the SIS-NET project, started in February 2025 upon approval by Ethical Committee (ID 5716), at the Department of Paediatrics, Paediatric Haematology, and Transplant Centre of Fondazione IRCCS San Gerardo dei Tintori (Monza). Nasopharyngeal swabs collected from all enrolled patients were analysed by the Microbiology Laboratory of IRCCS San Gerardo using the molecular assays: Alinity m Resp-4-Plex (Abbott) and Allplex Respiratory Panels (Seegene).

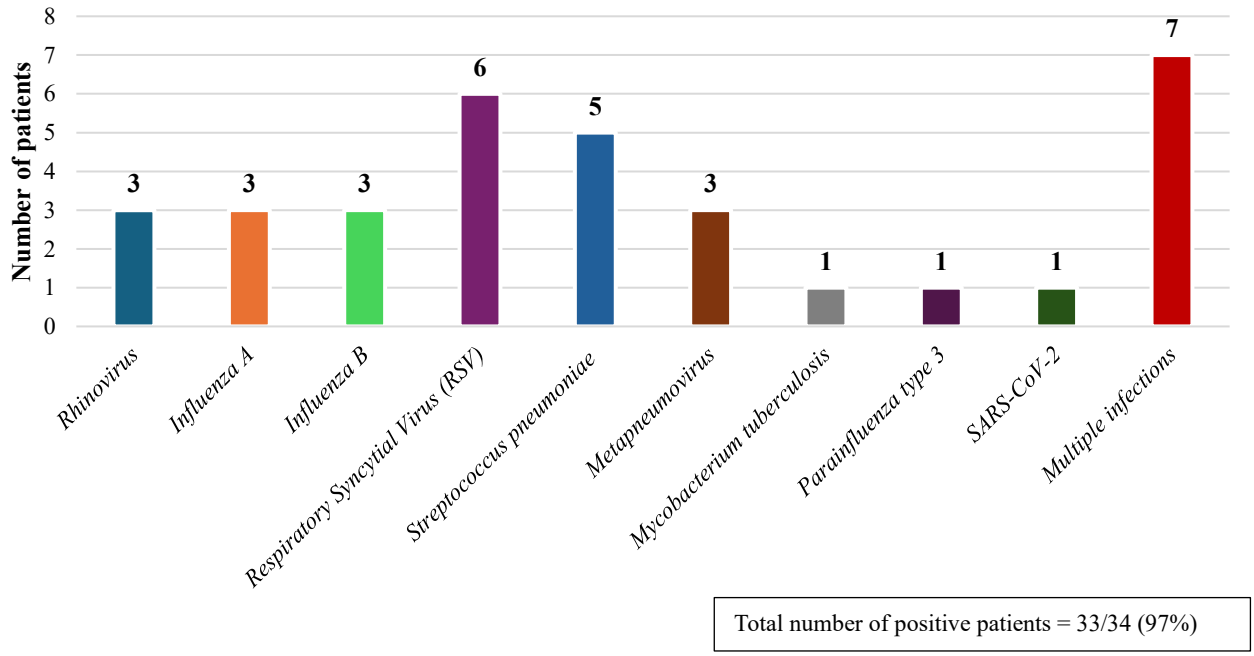
Results:

A total of 34 paediatric patients (18 males;16 females) have presently been enrolled, with a median age of 3.8 years (range 4 months -18 years). Among them, 13 (38%) have an underlying pathological comorbidity. Microbiological investigations were positive in 33 patients (97%) (Fig.1). Of these, 26 patients (76.5%) had single respiratory pathogen identified: RSV (n=6), *Streptococcus pneumoniae* (n=5), Rhinovirus (n=3), Influenza A (n=3), Influenza B (n=3), Metapneumovirus (n=3), *Mycobacterium tuberculosis* (n=1), Parainfluenza type 3 (n=1), SARS-CoV-2 (n=1). Multiple infections were identified in 7 of 34 patients (20.6%). Notably, one patient had both Influenza A and RSV, while another had *Mycobacterium tuberculosis* and Rhinovirus. Other pathogens involved in co-infections included *Haemophilus influenzae*, Adenovirus, and Bocavirus.

Discussion and Conclusions:

The preliminary results from the SIS-NET paediatric study have shown a wide range of viral and bacterial pathogens associated with community-acquired severe respiratory infections in children, often complicated by underlying conditions, underscoring the need for comprehensive diagnostics and personalized care. Future steps will include further characterization of the detected respiratory pathogens to better understand their characteristics and guide targeted interventions.

Figure 1



Total number of positive patients = 33/34 (97%)

347 - Rapid searches across million-genome bacterial collections on laptops: a practical methodology for point-of-care applications using k-mers

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Rapid searches across million-genome bacterial collections on laptops: a practical methodology for point-of-care applications using *k*-mers

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Introduction - Recent advances in sequencing technologies have led to the generation of massive collections of bacterial genomes, such as 661k (n=661,405) and AllTheBacteria (n=2,440,377), offering great potential for point-of-care (POC) applications, such as in rapid diagnostics and epidemiological surveillance. However, searching across millions of genomes in real-time on portable devices is challenging as existing approaches either search across limited genome subsets (e.g., BLAST), require computational clusters (e.g., BIGSI) or have prohibitive space and time requirements for laptops (e.g., LexicMap). Recently, alignments against these large collections have been made possible by Phylign leveraging the phylogenetic compression technique. However, Phylign's suitability for time-critical applications remains limited due to its reliance on COBS index, which is inefficient when searching divergent or long sequences, like plasmids. Although more modern *k*-mer indexes can be combined with phylogenetic compression, no established methodology exists to guide their selection, application, and parametrization. Therefore, the aim of this study is to define a methodology for *k*-mer-based searches across million-genome collections on standard laptops using phylogenetic compression, to address specific biological questions.

Materials and Methods - Our methodology guides the use of *k*-mer searches in 3 steps, as follows. 1) An initial biological question of interest is translated into a *k*-mer-based algorithm, 2) formalizing *k*-mer searches as a combination of 3 elements: query type, reference collection type, and matching mode. 3) This combination guides the selection of *k*-mer indexes to evaluate based on their indexing and searching performance, in order to identify the most suitable one for the given application. The whole methodology was applied to the problem of plasmid search using

Phylign and the 661k collection, comparing 4 modern *k*-mer indexes (COBS, Fulgor, Themisto and Metagraph).

Results - Different compression power and search speed characteristics were shown by the evaluated indexes. Metagraph offered the best compression power but the slowest plasmid searches. Themisto showed better performance on closely-related genomes. Fulgor offered the best trade-off, having similar index size to Metagraph and COBS while archiving the fastest plasmid searches. As a result, Phylign achieved ~4x faster plasmid searches when combined with Fulgor.

Discussion and Conclusions - Rapid searches across million-genome collections are crucial at POC. Our work provided the first practical guidelines for microbiologists to address their biological questions on a large-scale and on standard laptops using *k*-mer searches, paving the way for novel strategies at POC.

359 - Democratization of anti-infective biologicsthrough physics-informed generative AI

Vito Di Cioccio [(1)]

[(1)] *mAIbe S.r.l.*

Democratization of anti-infective biologics through physics-informed generative AI

Vito Di Cioccio – Co-founder and CEO of mAIbe S.r.l.

mAIbe is an innovative biotechnology startup leveraging physics-informed generative artificial intelligence to revolutionize discovery and development of monoclonal antibodies (mAbs) for infectious diseases. The company addresses the key limitations of traditional mAb discovery, which has historically relied on **trial-and-error** experimentation, an approach that is costly, time- consuming, and often lead to suboptimal results. Developing an anti-infective mAb typically takes **2 to 3 years**, requires up to **$50 million**, and has a **clinical success rate of just 10%**. Conventional technologies, including phage display, yeast display, and B-cell sequencing, depend on the prior circulation of a pathogen to isolate antibody candidates from recovered or vaccinated individuals and face limitations in keeping pace with antigenic mutation and evolutionary dynamics of infectious pathogens. These techniques often identify antibodies against immunodominant epitopes, which may not be functionally relevant or cross-protective. Moreover, current workflows rarely consider developability characteristics, such as stability, solubility, or expression yield, until late in the process, leading to high attrition rates and increased costs.

In contrast, **mAIbe** introduces an innovative paradigm: a shift **from empirical selection to a rational, design-based approach** powered by **physics-informed generative AI**. This computational method enables rapid de novo design and/or optimization of mAbs, against emerging pathogen variants. mAIbe's platform also improves affinity, stability, half-life, and developability of the generated mAbs, dramatically reducing development timelines and costs.

The core of **mAIbe's platform** is a tightly integrated computational–experimental cycle. AI-generated candidates are refined through physics-based simulations (e.g., molecular dynamics), ensuring that they are not only statistically plausible but also structurally robust. A **Lab-in-the-Loop** system supports iterative experimental validation (Design → Express → Test), where in vitro results are fed back into the AI models to retrain them and improve their predictive performance, ultimately increasing success rate.

By accelerating the design process, mAIbe's **integrated AI-Biotech approach** can respond rapidly to pathogen mutations and emerging variants, allowing timely modifications of mAbs. This could expand the accessibility of antibody therapies for infectious diseases, a field still underrepresented in the market, with only 17 out of more than 170 approved mAbs currently targeting infectious disease indications.

mAIbe differentiates itself by adopting a dynamic, end-to-end business model, rooted in strategic partnerships with academic and private entities (listed in the text). This collaborative network, combined with founders' expertise, enables mAIbe to cover the entire R&D process, **from discovery to clinical proof of concept (PoC)**. The end-to-end capability is a unique asset and a critical driver of mAIbe's competitive advantage.

4 - A Novel ARPE-19 Cell-Based Assay for Assessing Type I IFN Protection and Autoantibody-Mediated Neutralization in Zika Virus Infection

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Abstract SIM 2025

A Novel ARPE-19 Cell-Based Assay for Assessing Type I IFN Protection and Autoantibody-Mediated Neutralization in Zika Virus Infection

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Abstract:

Background: Type I interferons (IFNs) are critical mediators of the innate immune response against viral infections, including Zika virus (ZIKV). However, neutralizing autoantibodies (auto-Abs) targeting these IFNs can compromise their antiviral activity, potentially exacerbating disease severity.

Objective: This study aimed to develop and validate a sensitive in vitro assay using ARPE-19 cells to evaluate the protective efficacy of type I IFNs (IFN-α2, IFN-β, and IFN-ω) against ZIKV and assess the neutralizing capacity of patient-derived auto-Abs.

Methods: Using ARPE-19 (human retinal epithelial) and Vero E6 (monkey kidney epithelial) cell lines, cells were pre-treated with graded doses of recombinant IFNs and then infected with wild-type ZIKV (MR 766 strain). Patient sera were screened for IFN-neutralizing auto-Abs using ELISA and functional neutralization assays. The assay measured ZIKV replication and cytopathic effects as a readout of IFN protection or neutralization.

Results: ARPE-19 cells exhibited strong IFN sensitivity, showing inhibition of ZIKV replication at low IFN concentrations (≥10–50 pg/mL for IFN-β, ≥50 pg/mL for IFN-ω, ≥500 pg/mL for IFN-α2). In contrast, higher doses were required for Vero E6 cells. Importantly, sera containing autoantibodies against type I IFNs significantly impaired IFN-mediated antiviral protection, resulting in enhanced ZIKV replication in both ARPE-19 and Vero E6 cell lines.

Conclusion: The established ARPE-19-based assay effectively models the interplay between IFNs and neutralizing auto-Abs during ZIKV infection. This platform enables reliable detection of functional auto-Abs and holds potential for clinical applications in diagnostics and risk stratification, including for maternal and neonatal populations.

6 - Novel isolate of Halobacteriovorax capable of killing Multi-Antibiotic Resistant Escherichia coli and Salmonella

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Novel isolate of *Halobacteriovorax* capable of killing Multi-Antibiotic Resistant *Escherichia coli* and *Salmonella*

DONATELLA OTTAVIANI[1], SILVIA PIERALISI[1], GIULIA TALEVI[1], ELENA ROCCHEGIANI[1], FRANCESCA LEONI[1], MAIRA NAPOLEONI[1], DIEGO MAIOLATESI[1], FRANCESCA BARCHIESI[1], SARA NARDI[1] , GABRIELE ANGELICO[1], STEFANIA DILULLO[1]
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1. Introduction

The excessive and often inappropriate use of antibiotics in medicine, agriculture and other activities has facilitated the selection of Antibiotic-Resistant (AMR) pathogens, many of which are Multidrug-Resistant (MDR). The interconnection between humans, animals and their environmental ecosystems inevitably leads to the transmission of antibiotic resistance. This study aimed to isolate *Halobacteriovorax* from aquatic environment with predatory potential against MDR pathogens, particularly *Escherichia coli* and *Salmonella* of different origins.

2. Materials and Methods

The isolation of *Halobacteriovorax* was carried out in brackish water near the mouth of the Musone river (Marche region) using as primary prey a Multi-Antibiotic Resistant (MDR) strain of *Escherichia coli*. One *Halobacteriovorax* isolate, named HE7 was identified by PCR analysis performed on a fragment of the 16S rRNA gene of the *Halobacteriovoraceae* family members using the primers Bac676F and Bac1442R. Sequencing analysis was also performed on PCR product of 700 bp from a unique plaque. Moreover, A BLAST Search in NCBI GenBank was performed for 16S rRNA gene sequence of HE7 and compared to phylotypes and clones described for similar sampling sites.

The HE7 strain predation efficiency was tested against other MDR *E.coli* and *Salmonella* strains of different origin. Finally, HE7 strain was tested in challenge predator/ primary prey experiments to monitor predator/prey reduction at selected time points. Double-layer agar

plating technique was used to detect *Halobacteriovorax* and to evaluate its host specificity and predation efficiency.

3. Results

The 16SrRNA sequence of HE7 showed a 99,85% (671/672 bp) % identity to the 16SrRNA sequence of the strain F2 di *Bacteriovorax* sp (GenBank accession number AY294218). HE7 showed in vitro predatory activity against all MDR strains of *E. coli* and *Salmonella* tested. In the 10^8 predator/10^3 primary prey and 10^8 predator/10^8 primary prey challenges HE7, after 6 h, determined the total killing and 8 log reduction of the prey, respectively, maintaining this effect for up to 24 h.

4. Discussion and Conclusions

To date, exploration of predatory bacteria as an alternative to antibiotics has been mostly focused on *Bdellovibrio bacteriovorus*. To the best of our knowledge, this is the first work testing in vitro the efficacy of the genus *Halobacteriovorax* against MDR *E.coli* and *Salmonella* isolates. The results of this study highlight that HE7, but more generally *Halobacteriovorax* could find application alone or in an integrated context of antimicrobial strategies as alternative to antibiotics.

The study was supported by the Health Ministry financed projects RC 7/ 2023

8 - BacExplorer - A tool for denovo bacterial genome annotation

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BacExplorer - A tool for denovo bacterial genome annotation

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Introduction: Bacterial infections are a major global health challenge, causing millions of deaths each year. Traditional culture-based diagnostics are often slow and limited in scope, whereas molecular methods offer rapid, highly sensitive detection of both culturable and non-culturable pathogens. This advancement is especially critical in light of the growing threat of antimicrobial resistance (AMR), increasingly recognized as a modern-day "pandemic." Next-Generation Sequencing have become an integral part of routine analysis for microbiologists. These technologies provide profound insights into microbial diversity and evolution, accurately predict AMR genes facilitating timely decisions. However, the process of sequencing dozens of samples generates vast amounts of data that cannot be annotated manually. **Methods:** To address this challenge, numerous tools for bacterial genome analysis have been developed. These tools enable users to significantly accelerate their analyses permitting the identification of AMR and virulence genes within bacterial populations, along with other microbial characteristics. This genome annotation requires the integration of multiple specialized bioinformatics tools, a process that is both challenging and time-consuming. Each tool comes with its own strengths and limitations and often its proper usage requires intricate parameter tuning. Therefore, this complexity can overwhelm researchers. **Results:** To address this limitation, we created BacExplorer: a locally installable tool with an intuitive interface and

interactive HTML reporting, designed to enable users of varying expertise to perform analyses independently and efficiently. It performs comprehensive analyses on microbial genomes, including Multi-Locus Sequence Typing (MLST), antimicrobial resistance (AMR) profiling, virulence factor identification, and plasmid discovery. Additionally, for certain clinically relevant bacterial, genus- and species-specific structural analyses can further enhance diagnostic and research outcomes. For instance, *Klebsiella spp*. and *Escherichia coli* are analyzed employing Kleborate, this tool gives K (capsule) and O antigen (LPS) serotype prediction. Furthermore, scc-mec, agrvate and spatyper work on *Staphylococcus aureus* identifying respectively SCC*mec* casettes, *agr* locus and the *spa* type. Other tools permit the analysis of *S.pneumoniae*, *S.pyogenes,* and *L.pneumophila*. **Conclusions:** By streamlining the workflow, BacExplorer significantly improves access to advanced genome annotation, aiming to make high-throughput sequencing analysis a routine practice in laboratories. This would enables researchers to seamlessly integrate these insights into medical reports. BacExplorer is available at: https://github.com/knowmics-lab/BacExplorer.

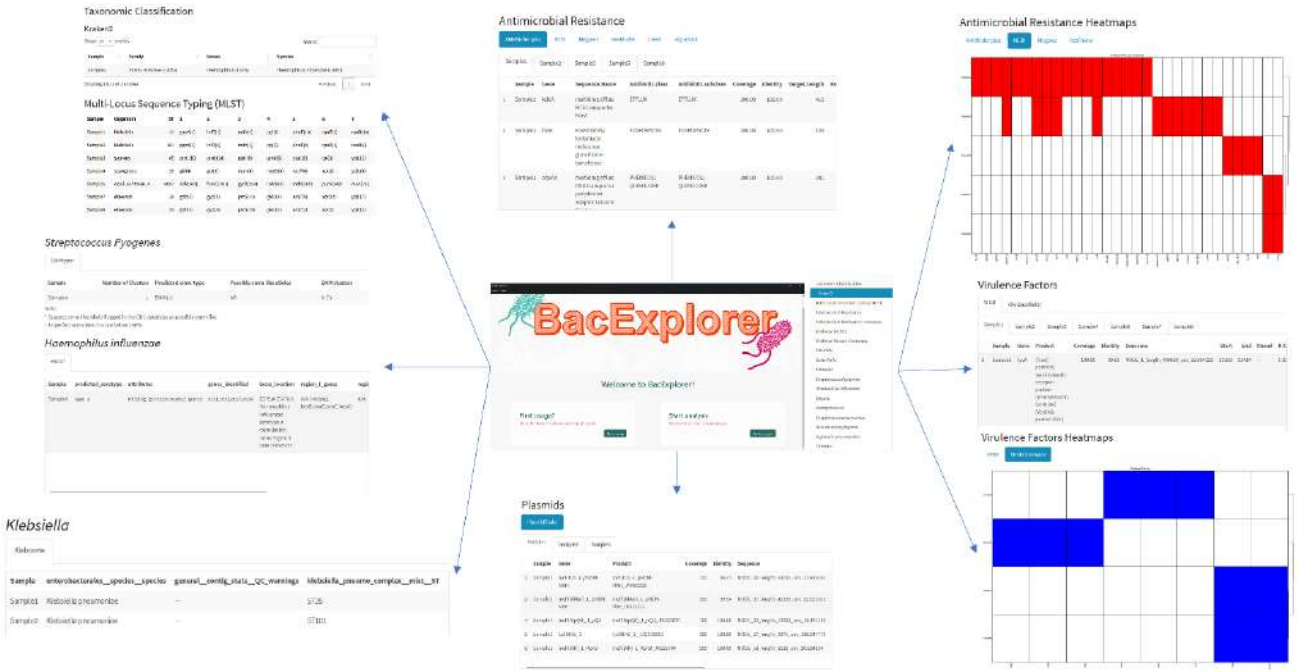

Fig. BacExplorer interface and HTML report screens.

15 - Development of a personalized whole blood assay to predict adverse reactions to antisense oligonucleotides for the treatment of viral infections

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Development of a personalized whole blood assay to predict adverse reactions to antisense oligonucleotides for the treatment of viral infections

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Introduction: Targeting viral nucleic acids with antisense oligonucleotides (ASOs) offers a promising strategy for the treatment of viral infections, addressing many of the limitations associated with traditional small molecule therapies. However, the potential of ASOs to trigger unintended immune responses remains a significant barrier to their use as antiviral agents. In this study, we developed and optimized a human whole blood *in vitro* assay to assess innate immune activation induced by various ASOs.

Materials and Methods: Initially, we investigated the relationship between clinical outcomes in mice treated with ASOs and cytokine responses elicited *in vitro* in ASO-stimulated mouse whole blood. We then compared these cytokine profiles to those observed in human blood to identify potential parallels.

Results: Through this approach, we identified *in vitro* cytokine signatures that correlate with the immunogenicity of ASOs found to cause adverse effects *in vivo* in mice. The human whole

blood assay was subsequently refined for improved reproducibility and sensitivity, and validated using samples from multiple donors to account for inter-individual variability. Our results demonstrate that ASO-induced cytokine responses are influenced by factors such as chemical structure, delivery method, and nucleotide modifications.

Discussion and Conclusions: The developed assay provides a predictive tool for early immunogenicity screening of ASOs in preclinical development, potentially reducing dependence on animal models and facilitating the design of safer therapeutic candidates. This platform may be valuable for both high-throughput screening and personalized therapy applications, particularly in the context of critically ill patients.

48 - Probiotic Alkalihalobacillus clausii strains and the gut microbiota: modulatory effects in an in vitro model of the human gut microbiota

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Probiotic *Alkalihalobacillus clausii* strains and the gut microbiota: modulatory effects in an *in vitro* model of the human gut microbiota

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Introduction: Culturing the human gut microbiota in *in vitro* models and testing on it different treatments *ex vivo* are arousing increasing interest in clinical research. We previously demonstrated that the addition of an enteropathogen to our 3D *in vitro* gut microbiota model negatively impacted intestinal microbial communities leading to dysbiosis. With the aim to evaluate the effects of beneficial microbes on the gut microbiota, in this study, four probiotic *Alkalihalobacillus clausii* strains were added in the *in vitro* model and the composition of the microbial communities was evaluated.

Materials and Methods: Mucin-coated electrospun gelatin membranes, biofabricated to simulate the three-dimensionality of the intestinal mucus layer, were used as scaffolds for microbial adhesion and growth in the *in vitro* model. The fecal microbiota, obtained from a healthy donor, was incubated for 24 h in the model to make microbial communities stable. Then, the four *A. clausii* strains were separately added (10^6 CFU/well) and co-incubated with the gut microbiota for 24 h, simulating the oral administration of probiotics. After 24 hours (T_1), incubation of half of the samples was stopped. For the remaining samples, supernatants were removed and replaced with equal volumes of fresh culture medium, and cultures were incubated for further 24 h (T_2). At T_1 and T_2, bacteria from membranes and suspensions were collected and subjected to genomic DNA extraction. 16S rRNA gene-targeting genus-specific Real-Time quantitative PCRs were performed to evaluate the composition of the microbiota in the presence and absence of *A. clausii*.

Results: After 24 h of treatment with *A. clausii* (T_1), no differences in the microbiota composition were evidenced compared to the untreated control. Surprisingly, significant increases in *Akkermansia* and *Faecalibacterium*, two microbial biomarkers of a healthy microbiota, were detected at T_2 for two of the tested *A. clausii* strains.

Discussion and Conclusions: The finding that probiotic *A. clausii* strains do not induce relevant modifications of the healthy gut microbiota *in vitro*, while exerting a positive effect on *Akkermansia* and *Faecalibacterium*, suggests that these probiotics can improve intestinal well-being in healthy conditions. Experiments evaluating the effects of *A. clausii* on a dysbiotic microbiota are ongoing. The results of this study could provide insights into whether

administering such probiotics can aid in recovering from dysbiosis commonly associated with intestinal infections and antibiotic consumption.

63 - Metagenomics Approach in Viral Pneumonia Diagnosis: Perspectives, Challenges, and Opportunities

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Metagenomics Approach in Viral Pneumonia Diagnosis: Perspectives, Challenges, and Opportunities

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Introduction

Lung infections are the most common and significant infectious diseases due to their high morbidity and mortality rates, particularly among elderly and immunocompromised individuals. In recent years, the number of threats caused by viruses has been steadily increasing. From a diagnostic and surveillance perspective, laborious differential diagnosis is often necessary. In recent years, next-generation metagenomics sequencing (mNGS) has been widely adopted for the detection of various infectious pathogens, showing, in some aspects, diagnostic performance better than traditional methods. In accordance with the above, the aims of this study were:

(i) Implementation of a metagenomics approach to analyse clinical samples collected from patients with clinical respiratory syndromes.

(ii) Comparison of mNGS performance with standard diagnostic virology methods

Materials and Methods

To date a total of 167 respiratory samples collected from patients with influenza-like illnesses or acute respiratory infections were tested with a multiplex real-time RT-PCR panel for respiratory viruses. MNGS sequencing was performed in both samples in which one or more viruses could be identified as well as those in which a pathogen could not be identified. In

briefly: total nucleic acids were extracted using an automated extraction platform and then splitted in two aliquots to construct separate DNA and RNA libraries. DNA and RNA libraries were sequenced on an Illumina platform. The obtained paired sequences were analyzed with the cloud-based metagenomic platform CZ ID.

Results

The mNGS protocol was performed on the 167 respiratory samples. The sequences obtained and analyzed were compared with the results of routine diagnostic tests to investigate the concordance between the two techniques. Out of the 167 samples, 122 (73,1%) exhibited concordance between the mNGS and standard diagnostic results (52 negatives and 70 positives). The discordance between the two approaches was observed in 45/167 samples (26,9%). In 7 samples, positive for a single virus, coinfections were detected; while in 12 samples, discordant pathogen identifications were observed. Furthermore, in 15 samples where no clinical diagnosis could be achieved, mNGS identified one or more pathogens compatible with the clinical syndrome. However, in 11 sample, mNGS failed to identify the pathogen.

Discussion and Conclusions

In conclusion, the preliminary data indicate that the mNGS protocol demonstrated satisfactory performance. Concordance with conventional tests was observed in 73,1% of cases. In 7 cases, mNGS helped in identifying coinfections, and in 15 cases, it provided a diagnosis that would have been impossible with traditional assays. When high levels of genomic coverage and sequencing depth are achieved using mNGS, it can be used for genetic and phylogenetic analyses to gather additional epidemiological information alongside viral identification.

64 - Neutrophil-derived biomarkers in serum samples of septic patients

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Neutrophil-derived biomarkers in serum samples of septic patients

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INTRODUCTION: Homer and then Hippocrates, Plutarch and Galen referred sepsis as a "putrefaction" of human tissues, which was the first step toward death. They had no idea about sepsis etiology and mechanism, however up to date we still lack an exact role of the hundreds mediators involved in sepsis pathogenesis. Even more clinically interesting should be the potential function of such mediators as sepsis biomarkers. The aim of this multicenter retrospective observational study is to evaluate serum levels of neutrophil born biomarkers such as calprotectin, azurocidin and IL-8 chemokine in patients with bacterial sepsis, comparing them with patients suffering from SIRS and healthy controls. **MATERIALS AND METHODS:** This study included adults (18–70 years) with confirmed bacterial sepsis and a qSOFA score of 1 or higher. Exclusion criteria included malignancies, substance abuse, pregnancy, and lactation. Serum levels of calprotectin, azurocidin and IL-8 were measured by biochip array technology (chemiluminescence, CTK HS kit – Randox) and ELISA (Calprest NG – Eurospital, AZU1 kit). Data were analyzed by ANOVA, with post-hoc significance using the PLSD test ($p < 0.05$). Results were expressed as mean ± SD for continuous variables and percentages for categorical data. The accuracy, sensitivity, and specificity of markers have been tested by ROC analysis. **RESULTS:** Between February and June 2024, 136 patients (74 males, 62 females, mean age 62.35 ± 17.67) with suspected sepsis and 15 healthy controls (mean age 37.53 ± 15.32) were enrolled. Procalcitonin (PCT) and C-reactive protein (CRP) levels were assessed as reference biomarkers. Based on ANOVA and ROC curve analyses, IL-8 and PCT exhibited very high diagnostic effectiveness but showed poor prognostic value, suggesting they could be useful for initial diagnosis, but not for predicting patient outcomes. In contrast, calprotectin and azurocidin exhibited fascinating characteristics, with significant differences in serum levels between SIRS and septic patients, and also substantial differences between septic survivors and septic non-survivors. These findings indicate that these biomarkers could serve as diagnostic and prognostic tools in sepsis management. Finally, CRP levels were significantly higher in septic non-survivors compared to survivors, though CRP could not differentiate between SIRS and sepsis, as its levels were similar in both groups. **DISCUSSION AND CONCLUSION:** In conclusion, calprotectin and azurocidin, emerge as promising biomarkers for diagnosing and predicting sepsis outcomes. These markers showed significant differences

between SIRS and sepsis and between septic survivors and non-survivors. While IL-8, and procalcitonin were effective diagnostically, their prognostic value was limited.

74 - Genetic analysis of Hepatitis C Virus in suspected iatrogenic transmission clusters: evidence from a healthcare-associated outbreak

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Genetic analysis of Hepatitis C Virus in suspected iatrogenic transmission clusters: evidence from a healthcare-associated outbreak

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Introduction: Hepatitis C virus (HCV) is an RNA virus that could cause chronic liver disease, potentially leading to cirrhosis and hepatocellular carcinoma. It is primarily transmitted via blood-to-blood contact, though iatrogenic transmission can occur in healthcare settings due to improper handling of contaminated materials or reuse of instruments. This study analyzed the genetic variability of the HCV NS3, NS5B, and NS5A genes in genotype 1b infected individuals to identify potential transmission clusters, in a recent outbreak of acute HCV. **Materials and Methods:** Plasma from 11 individuals involved in suspected iatrogenic transmission events (occurred between Jan-Dec 2024) was analyzed for drug resistance testing and phylogenetic analyses. Viral RNA was extracted with the QIAamp Viral RNA kit (Qiagen) and amplified by RT/PCR with homemade protocols. Next-generation sequencing was performed on MiSeq platforms (Illumina) using the Nextera XT DNA Library Kit. Resistance-associated substitutions (RAS) were analyzed by the Geno2pheno algorithm (https://hcv.geno2pheno.org). For each individual, three fasta sequences were produced using different cut-off (5%, 20%, 100%). Phylogenetic trees were constructed using the Maximum Likelihood method, with 1000 bootstrap replicates for NS3, NS5A, and NS5B genes by IQ-TREE software. Bayes evolutionary models were selected by ModelFinder software. Bootstrap values ≥99% were considered

significant. **Results:** The majority of individuals were male (18.2% females) with a median age of 69 years (IQR 61-75). The median log serum HCV-RNA was 5.4 [IQR 4.5–6.7] IU/mL at the time of HCV diagnosis. For 10 out of 11 individuals, HCV sequences were successfully obtained, with only one showing no amplification (with indeed a negative HCV-RNA at sampling). Interestingly, all people failed to amplify the initial portion of the NS5B gene (800 nucleotides). Phylogenetic ML trees confirmed the 1b genotype and showed a unique transmission cluster (bootstrap ≥0.99). Among the 30 sequences (using the different cut-off), the obtained evolutionary divergences were minimal, with the following mean p-distances: NS3 0.00128 (SD 0.00056), NS5A 0.00060 (SD 0.00033), and NS5B 0.00056 (SD 0.00032). High concordance of polymorphic mutations was found across coding regions, and no RAS were detected. **Discussion and Conclusions:** Phylogenetic analyses showed that all individuals were infected with a unique HCV genotype 1b strain. Aminoacidic concordance was identified in NS3, NS5A, NS5B genes, with the same initial NS5B amplification failure in all individuals. In addition, the low p-distance values supported the uniqueness of infection source, reinforcing evidence for an iatrogenic transmission cluster.

76 - Gut Microbiota analysis of patients with Phenylketonuria: Is it the first step for personalized medicine?

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Gut Microbiota analysis of patients with Phenylketonuria: Is it the first step for personalized medicine?

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Introduction: Phenylketonuria (PKU) is an inherited metabolic disorder. The phenylalanine accumulates in the blood and becomes toxic to the brain (gut-brain axis). Neurodevelopmental damage and behavioural problems are preventable by early diagnosis and dietary treatment. The specific diet is started in the neonatal period and followed life-long. Herein, we evaluated gut microbial community of PKU patients using a holistic approach. **Materials and Methods:** The observational study was conducted in one year. Fifty-six subjects were enrolled, including 32 patients with PKU, and 24 health subjects (CTRL). Bacterial DNA was extracted from faecal samples using QIAamp DNA Stool Mini Kit. DNA concentration was measured using Qubit dsDNA BR assay kit. The 16S rRNA V3-V4-V6 regions were amplified. Sequencing was performed by MiSeq Illumina® platform. The alfa-diversity was assessed by Simpson index using Mann-Whitney/Kruskal-Wallis. The beta-diversity was measured by PCoA, statistical significance was assessed using PERMANOVA test (p-value < 0.05). The taxonomy was assigned by SILVA database. Specific microbiota signatures were evaluated applying LDA LEfSe score > 2.0. **Results:** *Bacteroidetes* was the predominant phylum in PKU (70.0%), and CTRL (68.7%), followed by *Firmicutes* with very similar percentage. The *Firmicutes/Bacteroidetes (*F/B) ratio was <1 considering both cohorts. At family level, *Bacteroidaceae* (49.4%) and *Prevotellaceae* (7.4%) were more abundant in PKU group. The alfa-diversity showed a significant difference in PKU vs CRTL at phylum, genus and species levels, while beta-diversity displayed significant difference at phylum and species levels. PKU group showed high

prevalence of *Parabacteroides (P.) distasonis*, and less prevalence of *Faecalibacterium (F.) preusnitzii*, and butyrate producing bacteria at species level. **Discussion and Conclusions:** The F/B ratio was similar in both groups. The alfa-diversity decreased in subjects with PKU, and beta-diversity showed significant difference of microbial compositions between the two cohorts. The gut microbiota of PKU group diminished in butyrate-producing species, such as *F. preusnitzii,* considered a biomarker for health status. However, the high level of *P. distasonis* can regulate inflammatory markers and promote intestinal barrier integrity. Finally, PKU patients do not displayed dysbiosis. Patient's healthcare requires a multidisciplinary approach, including Paediatric, Dietitians and Microbiologists, to monitor their compliance with the crucial diet intervention as well microbial shifts. Even if gut microbiota analysis by Next-Generation Sequencing technology is not yet standard practice, integrating it into routine diagnostics could enhance personalized nutritional therapy, improving patient outcomes.

81 - Evaluation of intra-host genetic variability of human respiratory syncytial virus in infected hospitalized children

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Evaluation of intra-host genetic variability of human respiratory syncytial virus in infected hospitalized children

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Introduction: Respiratory syncytial virus (RSV) is a major cause of acute respiratory tract infections (ARTIs) in children. Despite its clinical impact, intra- and inter-host evolutionary dynamics remain poorly understood. This study aimed to assess intra-host RSV genetic variability in hospitalized children using whole genome sequencing (WGS).
Materials and Methods: Out of 132 children admitted to IRCCS Policlinico Foundation, 35 (25.6%) were RSV-positive and hospitalized; nine of them were randomly selected based on nasopharyngeal swabs collected at days 0 (T0) and 3 (T1). WGS was performed on Illumina MiSeq using QIAseq-xHYB Kit (Qiagen, Germany). RSV subtype was determined by phylogeny.

Mutations of potential phenotypic/epidemiological relevance were identified using RSVsurver tool. Genetic variation over time was assessed with the Quasispecies Distance tool (Galaxy platform). The ratio of non-synonymous to synonymous substitutions (dN/dS) was estimated trough CODEML (PAML). The study was funded by the European Union-Next Generation EU, Mission 4 Component 1, CUP 2022MNXLE7.

Results: All nine children (median [IQR] age 0.39 [0.23–0.70] years) had lower respiratory tract symptoms; seven (77.7%) remained symptomatic at T1. Eight (88.9%) were infected with RSV-A and one (11.1%) with RSV-B. WGS of N, G, F and M2 genes revealed amino acid substitutions linked to key viral functions. RSV-A harboured ten mutations at the same prevalence at T0 and T1: two in G (N178G, 25.0%; G195E, 12.5%), five in F (T122A, 37.5%; S377N, 25.0%; A103T, A518V, S276N, all 12.5%) and three in M2 (K52R, 25.0%; N174S, T180A, 12.5%). RSV-B showed four mutations (F, R209Q; N, V97I, T372A; M2, H147L) at both T0 and T1. Overall, a limited intra-host RSV-A evolution was found (median [IQR] whole-genome distance 0.006 [0.005–0.008]; dN/dS <1 across genes). On the other hand, analysing the specific genes, the G gene showed a higher genetic distance (G: 0.008 [0.001–0.011]; F: 0.006 [0.004–0.008]; M2: 0.006 [0.004–0.008]; N: 0.005 [0.004–0.007]; p=0.303) and dN/dS (G: 0.402 [0.384-0.701]; F: 0.071 [0.053-0.090]; M2: 0.025 [0.015-0.033]; N: 0.020 [0.017-0.023]; p<0.001). RSV-B showed a whole-genome distance of 0.0187, with the greatest variability in the G gene (0.031), compared to F (0.015), M2 (0.011) and N (0.017). The G gene dN/dS ratio was 1.948, indicating positive selective pressure, lower ratios were seen in N (0.410), M2 (0.307) and F (0.290).

Discussion and Conclusions: This preliminary analysis shows limited RSV evolution during the infection in hospitalized children, with stable mutations at key sites involved in viral entry and immune evasion. Notably, the G gene exhibited higher variability in both RSV-A and RSV-B, likely reflecting its role in immune escape.

120 - A Non-Interventional Diagnostic NGS-based Assay for Rapid Bacterial Identification in Sepsis Based on Next Generation Sequencing

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A Non-Interventional Diagnostic NGS-based Assay for Rapid Bacterial Identification in Sepsis Based on Next Generation Sequencing.

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1. Introduction

Sepsis is a life-threatening condition characterized by a dysregulated immune response to infection, resulting in organ dysfunction and high mortality. Conventional Standard of Care (SoC) diagnostic methods, such as culture-based techniques, often involve turnaround times of up to 72 hours, delaying the initiation of appropriate antimicrobial. Early pathogen detection and antimicrobial administration - ideally within the first 3 hours – represent the cornerstone of management. However, empirical antibiotic selection may be hampered by the increased emergence of antimicrobial resistance, leading to ineffective treatment and increasing likelihood of adverse outcomes. To address this challenge, timely and accurate pathogens identification and their resistance profiles is crucial. Next Generation Sequencing (NGS) technologies have rapidly emerged as a transformative tool for the rapid and comprehensive detection of microbial species. NGS enables direct microorganisms characterization from clinical samples, offering rapid and comprehensive insights into microbial infections and antimicrobial resistance (AMR).

We present PISTE™ (Pathogen Identification Technology), an NGS-based workflow designed for rapid pathogen detection and AMR profiling from whole blood samples of patients with bloodstream infections. PISTE™ workflow integrates a full-length 16S rRNA sequencing approach allowing fast (~6 hours) and accurate microbial species characterization, with a subsequent Metagenomic sequencing phase enabling AMR genes detection and resistance profiles prediction. This study primarily aimed to evaluate the diagnostic performance of PISTE™ workflow compared to "gold standard" SoC cultures in patients with sepsis. The study was partially supported by Xjenza Malta, grant R&I-2024-019L.

2. Materials and Methods

This prospective, multicenter, proof-of-concept phase IIa clinical study was conducted across four study sites in Athens (Greece) and in collaboration with The BioArte Limited (San Gwann, Malta). Patients recruitment occurred over 4 months. Inclusion criteria included: i) age ≥ 18 years, ii) written informed consent from the patient or a legally designated representative, iii) Intensive Cre Unit (ICU) admission with high suspicion of infection defined by the presence of at least one sign of qSOFA score (quick Sequential Organ Failure Assessment), and iv) clinical indication for blood culture. Exclusion criteria were prior antibiotics use, or pregnant and breast-feeding women.

For each recruited patient, 20 ml whole blood were drawn prior to antibiotic administration and inoculated into a culture flask (autoincubator, 37° C). After 6 hours of incubation, a 5.5ml aliquot was collected under sterile conditions and sent to The BioArte Limited (Malta) for NGS analysis, while the remaining sample monitored for microbial growth. Upon growth detection, the flask was subcultured on Columbia Sheep Blood Agar, followed by microbial identification and Antimicrobial Susceptibility Testing (AST) using the BD Phoenix™ M50 system. In case of any signal of bacterial growth was observed after 7 days, the flask content was aspirated and cultured to exclude slow-growing pathogens.

DNA was extracted from 0.5ml blood after 6 hours of incubation and analyzed via full-length 16S rRNA and metagenomic sequencing using Oxford Nanopore technology. Bioinformatic processing included quality filtering, taxonomic classification, and antimicrobial resistance gene detection. Human reads were excluded, and microbial reads were assembled and annotated against established databases. Diagnostic performance of PISTE™ was compared to standard-of-care cultures, evaluating sensitivity, specificity, predictive values, and overall accuracy using SPSS.

3. Results

A total of 100 patients were enrolled, with 71 declared sepsis-positives based on Sepsis-3 criteria. Pathogen classifications were adjudicated by two independent infectious disease experts using full clinical data to discriminate between true pathogens or putative contaminants: 14 patients were declared as true positives.

PISTE™ successfully identified pathogens in 13 of these 14 cases, yielding an overall accuracy of 95.7% [95% CI: 88% – 98.5%], sensitivity of 91.7% [95% CI: 64.6% - 98.5%], specificity of 96.5% [95% CI: 88.1% – 99%], PPV of 84.6%, and NPV of 98.2%. Shotgun sequencing was successful in 7 out of 13 positives; failures were linked to low microbial biomass (below detection limit). Antimicrobial resistance prediction showed 100% Accuracy for Cephalosporins and Penicillins (95% CI: 64.6-100%), 85.7% for Carbapenems (95% CI: 48.7-97.4%), and 71.4% for Aminoglycosides (95% CI: 35.9-91.8%). Lower predictive performance was observed for Tetracyclines and Quinolones resistances (57.1% Accuracy, 95% CI: 25-84.2%).

Secondary endpoint analysis revealed a statistically significant advantage of PISTE™ technology, with a computed *P* value < 0.0001. The average time to pathogen identification and

AST using PISTE™ was 12.0 hours, in contrast to the median of 30.4 hours (Q1-Q3: 28.9-33.7) for SoC cultures.

4. Discussions and Conclusions

The application of NGS in clinical settings is of great interest, particularly for time-sensitive conditions such as sepsis. NGS represents a valuable all-in-one tool for pathogen identification and antimicrobial resistance prediction. The PISTE™ workflow, based on Oxford Nanopore sequencing, demonstrates rapid microbial detection and prediction of antimicrobial resistance profiles from whole blood samples. The workflow integrates semi-automated DNA extraction following a 6h incubation and leverages full-length 16S rRNA alongside Metagenomics. In our study cohort, PISTE™ showed high diagnostic performance, achieving 95.7% accuracy, 91.7% sensitivity, and 96.5% specificity when compared to traditional SoC cultures. Additionally, the workflow effectively predicted resistance to several clinically important antimicrobial classes, although lower accuracy was observed for Tetracyclines and Quinolones.

This study highlights the potential of PISTE™ workflow as a rapid, hypothesis-free diagnostic platform for early detection of bloodstream infections and AMR genes.

By significantly shortening the time to pathogen identification and AST, the workflow has the potential to meaningfully change the clinical management and care pathway of patients with severe infections. These results support the broader implementation of NGS-based diagnostics supporting timely data-driven clinical decisions and precise targeted treatment strategies. As a next-generation diagnostic tool, PISTE™ has the potential to advance targeted infection management and contribute meaningfully to improved patient outcomes in critical care settings.

122 - Improving Prosthetic Joint infection diagnosis: direct MALDI-TOF identification from positive Bact/Alert inoculated with prosthetic sonication aliquots.

Mattia Genco *(1)* ***- Alessandro Bondi*** *(1)* ***- Marco Peradotto*** *(2)* ***- Fabio Longo*** *(1)* ***- Giorgia Piccinini*** *(3)* ***- Carlotta Polizzi*** *(1)* ***- Antonio Curtoni*** *(1)* ***- Cristina Costa*** *(1)*

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8. Diagnostica avanzata e innovativa max 2800 caratteri

Improving Prosthetic Joint infection diagnosis: direct MALDI-TOF identification from positive Bact/Alert inoculated with prosthetic sonication aliquots.

Mattia Genco[1], Alessandro Bondi[1,2], Marco Peradotto[2], Fabio Longo[1], Giorgia Piccinini[1,3], Carlotta Polizzi[1], Antonio Curtoni[1,2], Cristina Costa[1,2]

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1. Introduction

Prosthetic joint infection (PJI) is a complication of joint replacement that can develop into more serious consequences such as osteomyelitis and sepsis. A positive microbiological culture is an important data, fundamental for clinical diagnosis and treatment. In addition to the standard culture with different growth plates, an aliquot of sonication fluid can be inoculated into a BacT/Alert® bottle: this procedure has shown superior performances to standard culture, with high sensitivity.

2. Materials and Methods

We collected a total of 69 positive Bact/Alert from March 2024 to June 2024, inoculated with 65 prothesis aliquots after processing and 4 synovial liquids. The protheses were delivered in appropriate plastic boxes to the Microbiology and Virology Unit, Molinette Hospital (Turin, Italy).

We performed the sonication of the prothesis or its components. The sonication fluid (SF) was centrifuged, the supernatant was discarded, and the concentrated aliquots were plated on colombia nalidixin agar (CNA) and chocolate agar (CH) incubated at 37°C, 5% CO2 for 5 days and anaerobe blood agar incubated 37°C for 10 days in anaerobic conditions. Similarly, synovial liquid (SL) was centrifugated and the sediment was plated on CNA agar and CH agar incubated at 37°C, 5% CO2 for 5 days. Both concentrated aliquots of SF and SL were inoculated in Bact/Alert® and incubated for 10 days in BacT/Alert® VIRTUO® (bioMerieux, Marcy-l'Étoile, France). In case of BacT/Alert positivity we used Sepsityper kit to extract the bacterial sediment, and we performed MALDI-TOF mass spectrometry with Microflex (Bruker, Germany) to identify the bacteria.

3. Results

In 37 samples the direct cultures showed bacterial growth before VIRTUO positivity signal. On the other hand, 32 direct cultures resulted negative with positive vials. 51 cases developed a monomicrobial culture, while 18 had more than one bacterial species. The main isolates were Gram-positive, *S. aureus* and other *Coagulase-negative Staphylococci*. Considering MALDI-TOF identification, we divided the results in 3 groups. Group 1 consists of 33 samples (all SF): MALDI-TOF identification reached at least 2.00 score and matched to the culture. 16 samples belong to group 2 (15 SF and 1 SL) with results between 1,70 and 2.00 score and had 88% correlation MALDI-TOF/culture ID. In group 3 (3 SF and 1 SL), with score lower than 1,70 we had two *P. aeruginosa* with MALDI-TOF/culture concordance. For one sample MALDI-TOF identified only one species, while culture isolated three different bacteria. For the last sample MALDI-TOF ID had very low score.

4. Discussion and Conclusion

The addition of BacT/Alert to the standard inoculation protocol improved sensitivity performances and reduced the time to diagnosis of PJI. In addition, the use of Sepsityper kit allows to directly identify the bacteria before the sub-culture and to choose a more appropriate antibiotic therapy more quickly.

123 - Selection of highly specific nanobody against KPC-3 beta-lactamase: Toward improved diagnostic strategies

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Selection of highly specific nanobody against KPC-3 beta-lactamase: Toward improved diagnostic strategies

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1. Introduction:

Nanobodies (VHHs) have emerged as promising diagnostic and therapeutic tools owing to their unique physicochemical properties, which confer key advantages over conventional antibodies, particularly in infectious diseases. The global spread of Klebsiella pneumoniae carbapenemase-3 (KPC-3)–producing bacteria, a clinically relevant class A beta-lactamase that confers resistance to a broad spectrum of beta-lactam antibiotics, including last-resort carbapenems, emphasizes the urgent need for innovative diagnostic solutions. In this study, we aimed to isolate and characterize VHHs specific to the KPC-3 enzyme from an immune library, using phage display technology.

2. Materials and Methods:

A dromedary was immunized with increasing concentrations of purified KPC-3 enzyme. Peripheral blood lymphocytes were isolated, and cDNA was synthesized to construct a KPC-3-targeted VHH library. Selection of highly specific VHHs was performed through three rounds of biopanning using phage display. Individual clones from the enriched library were screened via enzyme-linked immunosorbent assay (ELISA) and sequenced. A selected nanobody displaying a unique VHH sequence was expressed, purified, and characterized to assess its specificity and binding capacity toward KPC-3, using ELISA and Western blot analysis.

3. Results:

We successfully constructed a VHH immune phage library with a size of 3.4×10^6 CFU/mL, in which more than 90% of the clones contained VHH inserts of the correct size. Following three rounds of biopanning, several clones exhibited strong ELISA signals, indicating high-affinity binding to the KPC-3 enzyme. Sequence analysis revealed a unique VHH sequence

characterized by hallmark amino acid residues typical of functional nanobodies. The selected nanobody was expressed in the *E. coli* WK6 strain and purified using HisTrap HP affinity chromatography. Purity of the nanobody was assessed by SDS-PAGE, which showed a single band at the expected molecular weight and was further confirmed by Western blot. ELISA validation demonstrated specific recognition of KPC-3, and the nanobody exhibited a high binding affinity, with an EC_{50} value of 4.3×10^{-11} M. Western blot analysis further confirmed its ability to detect KPC-3, reinforcing its potential for diagnostic applications.

4. Discussion and Conclusions:

The identification and characterization of a VHH with high specificity and binding ability toward KPC-3 supports its promising application in diagnostic platforms for beta-lactamase-producing pathogens. Its capacity to detect KPC-3 at low concentration makes it suitable for rapid, sensitive diagnostic tools essential in clinical settings for guiding antimicrobial therapy. This work lays a solid foundation for further studies, including functional assays and structural analysis to elucidate its binding mechanism, thereby contributing to the development of nanobody-based strategies to detect and combat resistance mediated by β-lactamase-producing bacteria.

124 - Fast and automated detection of common carbapenemase genes using multiplex real-time PCR in rectal swabs

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Fast and automated detection of common carbapenemase genes using multiplex real-time PCR in rectal swabs

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Introduction Antimicrobial resistance (AMR) represents a significant challenge to public health. Projections indicate that AMR may become the leading cause of mortality worldwide, with Italy exhibiting one of the highest rates in Europe. A cornerstone in contrasting AMR is the implementation of active surveillance, aimed at identifying carbapenemase-producing Enterobacterales (CPE) or other Gram-negative organisms (CPO) through rectal swabs. The conventional approach involved culture testing; however, molecular methods detecting carbapenemase genes show considerable promise due to shorter turn-around time and enhanced sensitivity. We aimed to assess the proficiency of REALQUALITY (RQ) Carba-Screen kit (AB ANALITICA) combining DNA extraction and RT-PCR assay within a single instrument, from rectal swab to detect KPC, OXA-48, NDM, VIM, and Acinetobacter OXA.

Materials and methods A total of 274 rectal swabs, collected from patients hospitalized in Padua University Hospital, were subjected to screening for CPO utilizing culture on agar plates for selective isolation of carbapenem-resistant Gram-negative bacteria as the gold standard. Results were compared with those obtained with RQ Carba-Screen kit. Discordant findings were analysed using an in-house RT-PCR.

Results Out of 274 rectal swabs analysed using RQ Carba-Screen kit, there were 5 invalid results even after sample dilution due to inadequate internal control amplification. Among the 269 correctly analysed samples, 26 (9,7%) tested positive with the Carba-Screen method, whereas culture identified 21 positive samples (7,8%). The Carba-Screen kit identified 32

targets (4 samples exhibited multiple genes): 12 KPC, 9 Acinetobacter OXA, 6 NDM, 3 OXA48, 2 VIM, while culture isolates resulted positive for 24 carbapenemase genes (namely 12 KPC, 5 Acinetobacter OXA, 4 NDM, 2 OXA48, 1 VIM, since 2 samples had multiple genes). The sensitivity of the Carba-Screen kit sensitivity was 100% while specificity was 98%. The 5 discordant results were subjected to further investigation via an in-house RT-PCR for detection of carbapenemase genes: in 4 out of 5 samples, the gene previously found in Carba-Screen kit test was detected, indicating a superior sensitivity of Carba-Screen kit compared to culture method.

Discussion RQ Carba-Screen kit demonstrated excellent sensitivity and specificity in detecting CPO directly from rectal swabs. Its limitations encompass a reduced capacity to process a high number of swabs in comparison to culture methods, and inherent inability to identify carbapenem-resistant Enterobacterales not producing carbapenemases. Further investigations are warranted to elucidate the implications of Carba-Screen kit technology on patient management and CPO epidemiology within large hospital settings.

131 - The bile microbiota composition in pathological conditions: a pilot study

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The bile microbiota composition in pathological conditions: a pilot study

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Introduction: The bidirectional crosstalk between the liver and gut is anatomically represented by the biliary tract, portal vein, and, more generally, systemic circulation. This leads to the assumption that even bile could be colonized by permanent microbiota that may be dysregulated and interfere with the patient's clinical outcome. In this pilot study, we sequenced human bile samples of patients with naive papilla of Vater which underwent endoscopic biliary

drainage to test the hypothesis of permanent microbiota and the existence of bacterial biomarkers in different pathological conditions. Material and Methods: A homogenous population of 48 human bile samples was included in the study, twenty-four patients had benign biliary obstruction (11 women and 13 men), and 24 had malignant jaundice (9 women and 15 men). The microbial DNA population was extracted with Maxwell® RSC Pathogen Total Nucleic Acid Kit and sequenced on the P2 solo device (Oxford Nanopore Technologies, Oxford, United Kingdom) by targeting the genes 16S and 18S. The community composition, the alpha and beta index, and richness diversity were calculated with One Codex (One Codex Inc., Wilmington, Germany). The reads count was adjusted by subtracting the reads sequenced in the negative control. A threshold of 1000 reads was set to exclude false positive results. Results: Once sequenced, 12 patients with a benign biliary obstruction (BBO) and 14 patients with a malignant biliary obstruction (MBO) had a positive biliary result. The threshold of 1000 reads resulted in valid to distinguish between positive and negative samples. Considering genera present in more than 2 patients, BBO and MBO shared 7 common bacterial genera. On the contrary, Aggregatibacter, Bacteroides, Campylobacter, Capnocytophaga, Clostridium, Neisseria, Oribacterium, and Porphyromonas were found only in BBO, and Helicobacter, Megasphaera, and Staphylococcus were sequenced only in MBO. Similarly, common genera between female and male populations were: Escherichia, Gemella, Granulicatella, Haemophilus, Neisseria, Prevotella, Streptococcus, and Veillonella. By linking the two databases: Porphyromonas, Capnocytophaga, Oribacterium, and Aggregatibacter were reported only in BBO women, whereas Megasphaera was associated with MBO women. Concerning the male population, Bacteroides and Clostridium were sequenced only in BBO men, and Staphylococcus in MBO men. The average Shannon index for positive BBO and MBO was 3.48 and 3.04, respectively. Discussion and Conclusion: The definition of the bile microbiota and its role in pathological conditions may represent a new perspective for the knowledge and treatment of biliary tract disorders. Some genera can be found only in female.

137 - Enhanced accuracy in HIV-1 subtyping through near full-length sequencing: a comparative analysis with commonly used methods

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Enhanced accuracy in HIV-1 subtyping through near full-length sequencing: a comparative analysis with commonly used methods

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Introduction. HIV-1 exhibits remarkable genetic diversity, driven by multiple evolutionary mechanisms occurring in infected individuals. Accurate subtyping of HIV-1 is essential for effective disease surveillance. Next-generation sequencing (NGS) is progressively replacing Sanger for genotypic drug resistance testing, in the protease/reverse transcriptase (PR/RT) and integrase (IN) regions. To assess the phylogenetic accuracy of subtype determination, near full-length (NFL) sequences obtained through NGS were compared with NGS-derived PR/RT and PR/RT/IN (pol) sequences, as well as with the output of four commonly used automated subtyping tools.

Material and methods. Thirteen plasma samples from people with HIV-1 were first sequenced using NGS with the Ion AmpliSeq panel for Ion GeneStudio S5 Prime System (Thermo Fisher), targeting the *pol* region (nt 2253–5250 in the HXB2 reference genome). The same samples were then sequenced using the Ion AmpliSeq Whole Genome Sequencing HIV-1 custom panel (nt 1–9719) to obtain NFL sequences. Phylogeny was conducted using MEGA v11 and the most up-to-date HIV-1 reference sequences. Three phylogenetic trees, based on PR/RT, *pol*, and NFL sequences, were constructed to determine subtypes. In parallel, the PR/RT, *pol*, and NFL sequences were analyzed using four automated subtyping tools: COMET v2.4, REGA v3, Geno2Pheno v3.5 (G2P), and Stanford v9.8. Subtyping results from these tools were compared to phylogeny.

Results. Among the subtyping tools, Stanford showed the highest concordance with PR/RT-based phylogeny (92.3%), followed by COMET, REGA, and G2P (each 61.5%). Using *pol* sequences, Stanford achieved full concordance (100%) with phylogenetic results, followed by COMET and G2P (both 69.2%), and REGA (46.1%). With NFL sequences, Stanford again demonstrated the highest concordance (92.3%), followed by COMET (61.5%) and REGA

(38.5%); G2P was unable to process NFL sequences. Phylogeny revealed reclassification of three samples into more complex recombinant forms: CRF02_AG to CRF06_cpx, G to CRF02_AG, and F1 to CRF46_BF1. All pure subtypes were correctly identified by the subtyping tools, except for REGA, which misclassified A6 samples as A1.

Discussion and Conclusions. Phylogeny remains the gold standard for accurate HIV-1 subtyping. However, in settings where it is not feasible for routine use due to its complexity, subtyping tools provide a practical alternative. Discrepancies among tools are common, largely due to inconsistent algorithm updates. Notably, this study underscores the importance of increased sequence length, showing that longer nucleotide sequences yield more consistent and accurate subtype classifications. This has critical implications for tracking viral diversity and identifying emerging recombinant forms.

142 - Critical Assessment of MetaProteome Investigation 2 (CAMPI-2): Multi-laboratory assessment of sample processing methods to stabilize fecal microbiome for functional analysis

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Critical Assessment of MetaProteome Investigation 2 (CAMPI-2): Multi-laboratory assessment of sample processing methods to stabilize fecal microbiome for functional analysis

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1. Introduction

Fecal samples are widely used as a proxy for studying gut microbiome composition in both human and animal research. Fecal metaproteomics provides valuable insights by tracking changes in the relative abundance of microbial taxa and their protein functions. To ensure reliable results, it is crucial to minimize alterations in the metaproteome occurring from sample collection to protein extraction. Therefore, employing effective stabilization methods is essential to preserve the integrity of the fecal metaproteome from sample collection to laboratory analysis, particularly over long distances or when rapid freezing options are not readily available. In line with these needs, the second edition of the Critical Assessment of MetaProteome Investigation (CAMPI-2) was specifically focused on improving sample stabilization prior to metaproteomic analysis.

2. Materials and Methods

This collaborative multicenter study assessed the ability of five different stabilization methods, based on two commercial devices and three specific reagents (acetone, lithium dodecyl sulfate, and an RNAlater-like buffer), respectively, to stabilize the fecal metaproteome during room-temperature storage (14 days) and shipment to mass spectrometry facilities. The five methods were tested simultaneously by eight different laboratories across Europe, using aliquots from the same fecal sample. After protein extraction and digestion, duplicate aliquots of the resulting peptides were analyzed independently by two mass spectrometry facilities at distinct international locations.

3. Results

Analysis of the mass spectrometric data using two different search engines revealed that the fecal metaproteome profile differed considerably depending on the stabilization method used in terms of richness, alpha and beta diversity, reproducibility, and quantitative distribution of main taxa and functions. Although each method showed unique strengths and weaknesses, a commercial swab-based device stood out for its remarkable reproducibility and ranked highest for most of the metrics measured.

4. Discussion and Conclusions

CAMPI-2 allowed a robust evaluation of five different methods for preserving fecal metaproteome samples. The present investigation provides useful data for the design of metaproteomics and multi-omics studies where fecal sampling cannot be immediately followed by long-term storage at −80°C. Further optimization of the tested protocols is necessary to improve stabilization efficiency and control bias in the taxonomic and functional profile of the gut microbiome.

150 - Accelerate Pheno™: Performance Evaluation in the Rapid Microbiological Diagnosis of Sepsis in the Intensive Care Unit of the University Hospital of Palermo

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Accelerate Pheno™: Performance Evaluation in the Rapid Microbiological Diagnosis of Sepsis in the Intensive Care Unit of the University Hospital of Palermo

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Introduction

Bloodstream infections (BSIs) represent a persistent and growing public health challenge. Timely and accurate identification of the causative pathogens, along with antimicrobial susceptibility testing (AST), is crucial for guiding targeted therapy and limiting the spread of multidrug-resistant (MDR) strains. To meet this need, several innovative diagnostic tools have been developed, including the Accelerate Pheno™ System (AXDX) an automated platform that performs both pathogen identification (ID) and AST of common bacterial pathogens directly from positive blood culture samples within approximately 7 hours. This study aimed to evaluate the performance of the AXDX system for the rapid diagnosis of sepsis at the A.O.U.P. P. Giaccone University Hospital in Palermo.

Materials and Methods

Between June 2022 and December 2024, 62 positive blood culture samples collected from Intensive Care Unit patients were analyzed. Samples underwent both conventional and rapid diagnostic workflows. Initial Gram staining was performed to exclude polymicrobial samples and yeasts from the rapid testing protocol. The conventional workflow included microbial identification using the MALDI Biotyper and AST with the BD Phoenix (PHX) system. The rapid workflow used the AXDX system.

Results

Of the 62 samples analyzed with the AXDX system, 50 monomicrobial positive blood cultures were correctly identified, with only one showing a discrepancy between the rapid and conventional methods. Seven samples were polymicrobial; among these, three exhibited discordant results upon culture. Of the five samples that did not yield an ID result, two discrepancies were attributed to the absence of the microorganism in the AXDX panel. Regarding AST, four samples did not produce results despite correct identification. Among the successfully tested samples, three categorical discrepancies were observed: two for Klebsiella pneumoniae with ceftazidime/avibactam and one for Acinetobacter baumannii with colistin. These discrepancies were further evaluated by E-test, which confirmed the accuracy of the PHX system.

Conclusions

There is an increasing need in clinical microbiology to integrate accurate MIC determination into rapid diagnostic technologies, especially in environments with a high prevalence of antimicrobial resistance. However, careful validation of the accuracy and clinical applicability of these rapid systems remains essential, particularly when dealing with MDR strains.

153 - Early antibiogram reading of Microscan Autoscan-4 System with MIC panel 2: preliminary results on MDR well-characterized Klebsiella spp. clinical isolates

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Early antibiogram reading of Microscan Autoscan-4 System with MIC panel 2: preliminary results on MDR well-characterized *Klebsiella* spp. clinical isolates

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Introduction. In clinical microbiology the rapid availability of antibiotic susceptibility results (ASTs) is crucial to guide/confirm a therapeutic choice. This study aimed to assess the performance of the panels rapid reading by both Microscan Autoscan-4 system (AS4, Beckman Coulter) and manually reading to reduce the turning around time on multidrug-resistant (MDR) *Klebsiella* spp.. **Materials and Methods.** Twenty well-characterized *K. pneumoniae* strains belonging to 20 MultilLocus Sequence Types, plus a single *K. oxytoca* isolate, were subjected (in duplicate) to susceptibility test using the MIC panel 2 of AS4. The resistome of the 21 isolates included: *bla*CTX-M-, *bla*SHV-, *bla*KPC-, *bla*VIM- type, *bla*OXA-48, *bla*NDM-1, *sul1*, *aac(6')-Ib*, *qnrS*, *qnrBcatB3*, *armA*, *mphE*, *msrE*, and *oqxA/B* genes. MIC panels, prepared according to manufacturer's instructions, were incubated at 35±2°C T. Subsequently, panels were read both manually and by the AS4 after five and six hours. The resulting profiles were compared to the reference reading at 18 ± 2 hours and EUCAST 2025 guidelines for blood stream infections. Nineteen antibiotics were evaluated: amikacin, amoxicillin/clavulanic acid (AMC), ampicillin (AMP), aztreonam (AZT), cefepime, cefotaxime (CTX), ceftazidime/avibactam (CZA), ceftazidime, ceftolozane/tazobactam (C/T), cefuroxime (CRM), ciprofloxacin, ertapenem, gentamicin, levofloxacin, meropenem (MP), meropenem/vaborbactam,

piperacillin/tazobactam (TZP), ticarcillin, tobramycin (TO), trimethoprim/sulfamethoxazole. **Results.** Preliminary readings obtained by AS4 after five hours of incubation showed categorical agreements (CA) ranging from 19% for TZP to 90.5% for both AMP and CZA. CA increased to 42.9% for TZP and 100% for AMP at six hours. Manually readings at five hours showed CA of 76.2% for TZP and MP, of 100% for aminoglycosides, AMC, C/T, AMP, AZT, CTX, CRM, and TO. AS4 readings after six hours showed the same performance as five hours for TZP and AMP, and an increase in CA for all antibiotics, except CZA. Manually reading at six hours showed the same CA as at five hours for all antibiotics, except for TZP (showing a 4.8% increase). Overall, the very major errors (VMEs; S/R) decreased from 26.6% at five hours to 13.8% at six hours by AS4 readings. Conversely, the VMEs were restricted to 2.3% and 1.7% at five and six hours, respectively, with manually reading. **Discussion and Conclusions.** These preliminary data showed the reliable role of manually readings in anticipating the ASTs and particularly the resistant phenotypes just after six hours' incubation. On the basis of the above promising results, and to further validate the reproducibility of the data, additional MDRO strains will be studied.

192 - The potential of metagenomic sequencing (mNGS) in the diagnosis of bacterial meningoencephalitis

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The potential of metagenomic sequencing (mNGS) in the diagnosis of bacterial meningoencephalitis

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Introduction

Central nervous system (CNS) infections – such as meningitis, encephalitis, and meningoencephalitis – are critical medical emergencies due to their rapid progression and potentially severe outcomes. The broad spectrum of causative pathogens and nonspecific symptoms often complicate diagnosis, which typically relies on cerebrospinal fluid (CSF) analysis through conventional culture and PCR-based molecular methods. However, these approaches may fail to detect fastidious, non-culturable, or unexpected pathogens. In this context, metagenomic Next-Generation Sequencing (mNGS) is emerging as a promising unbiased technique capable of detecting a wide range of microbial agents in a single assay. This study aimed to evaluate the diagnostic performance of 16S-targeted mNGS on CSF samples from patients with suspected bacterial CNS infections, comparing its results to those of routine microbiological methods used in the Microbiology and Virology Unit at the University Hospital of Sassari.

Materials and Methods

We retrospectively analysed 31 residual CSF samples collected between September 2022 and May 2024. Samples were stored at -20°C after completion of conventional testing. Thawed samples underwent automated nucleic acid extraction. Metagenomic analysis targeted the 16S rRNA gene V4 hypervariable region using Illumina sequencing. Bioinformatic analysis was carried out using the DRAGEN Metagenomics Pipeline on the Illumina BaseSpace platform; statistical processing was performed using XLSTAT2024®.

Results

mNGS outperformed traditional methods in sensitivity and specificity. It led to a diagnostic revision in 17 of 31 cases (55%). In five culture-positive samples, mNGS enabled their interpretation as skin commensal contaminants, being undetected in the corresponding CSF. In three clinically relevant confirmed cases of infective meningoencephalitis (IM) with negative cultures and PCR tests, mNGS identified bacterial pathogens (e.g., *Fusobacterium naviforme* and *Neisseria mucosa*). Seven taxonomic corrections were allowed by mNGS where MALDI-TOF misidentified bacterial species. Furthermore, two patients clinically reported as negative for bacterial infections were reviewed as IM thanks to mNGS detection of specific pathogens.

Discussion and Conclusions

This study highlights mNGS as a highly informative, unbiased diagnostic tool capable of detecting non-culturable or unexpected pathogens, improving diagnostic accuracy in CNS infections. Notably, mNGS may be particularly valuable in samples from patients early treated with antibiotics or with low pathogen load. When interpreted within the clinical context, mNGS offers substantial added value over standard methods and may help guide more targeted treatment in complex or unresolved cases.

194 - Socioeconomic position, multimorbidity and the mediatory potential of the gut microbiota

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Socioeconomic position, multimorbidity and the mediatory potential of the gut microbiota

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Introduction

People are living longer, but healthy life expectancy is not improving as much due to chronic diseases and multimorbidity, especially in older adults. Socioeconomic position (SEP), which reflects a person's social and economic status, plays a key role in health inequalities and is linked to chronic disease and mortality. However, the relationship between SEP and multimorbidity, and the role of lifestyle, biological factors, and working conditions, remains poorly understood. An observational study involving two Italian cohorts aims to investigate the association between SEP and multimorbidity, exploring the potential of microbiological and behavioral mediators.

Materials and Methods

The study uses data from the Lazio Region Longitudinal Study, including over 5.5 million residents followed from 2011 to 2022 through health databases on mortality, hospital admissions, drug prescriptions, chronic disease exemptions, and socioeconomic indicators. It also includes the European Prospective Investigation into Cancer and Nutrition-Turin (EPIC-Turin) cohort, with 10,604 participants recruited between 1993 and 1998 and followed for over 20 years. A sub-cohort of 500 participants will be recalled to update lifestyle, dietary, and sociodemographic data and to collect blood and stool samples. Blood is analyzed for metabolic, inflammatory, and DNA methylation profiles using Illumina Infinium MethylationEPIC arrays. Stool samples undergo 16S rRNA gene sequencing and metaproteomics.

Results

The Lazio database is being updated to include standardized indicators of SEP and multimorbidity. The EPIC-Turin recall phase is ongoing, with over 400 subjects recruited and interviewed, and 330 fecal samples collected up to date. Laboratory analyses of blood (whole genome methylome) and stool samples (gut microbiota profiles) are expected to be completed by September 2025. Expected results include the identification of associations between SEP

and multimorbidity, quantification of the mediating effects of lifestyle, biological aging, and gut microbiota, and assessment of the contribution of occupational conditions.

Discussion and Conclusions

This study will help clarify how SEP influences multimorbidity through biological pathways, including the roles of epigenetic aging and gut microbiota. The results are expected to support public health strategies aimed at early risk identification and the promotion of healthy ageing in socially and economically disadvantaged populations.

203 - MICROBIOLOGIST IN YOUR POCKET: EVALUATION OF COMMON AI SERVICES FOR GRAM INTERPRETATION

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MICROBIOLOGIST IN YOUR POCKET: EVALUATION OF COMMON AI SERVICES FOR GRAM INTERPRETATION

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INTRODUCTION

Image interpretation is still crucial for some laboratory diagnostics in clinical microbiology.

For example, the discriminatory observation of gram stains is included in the microbiological diagnostic flowchart of blood cultures for rapid support to therapy escalation/de-escalation. However, gram stain interpretation requires time and highly trained staff.

The purpose of our work was to evaluate the performance of AI in gram stains interpretation, in view of a possible integration in the clinical microbiology diagnostic.

MATERIAL E METHODS

A total of 128 Gram stains were collected and saved in jpeg format with a resolution of 1440x1440ppi. Specifically, we collected the images of 71 Gram negative strains, 49 Gram positive strains, 6 mixed Gram stains and 2 Candida strains.

Subsequently the images were analyzed with the 3 most used AI services: Chat GPT (Open AI), COPILOT (Microsoft) and GEMINI (Google).

The Analysis was conducted submitting the same prompt in Italian: " *Ti invierò una serie di fotografie di colorazioni di gram. Indica se i batteri visibili in questa colorazione di Gram sono*

Gram-positivi o Gram-negativi, specifica la forma (cocchi, bacilli, ecc.) e prova a indicare una specie presunta." All three AI services responded in "standard thinking" (4.0 GPT version). Finally, the performances of the three AI services were compared to human interpretation for Gram and morphology, considering "wrong" only major mismatches (e.g. cocci instead of bacilli and vice versa).

RESULT

For Gram identification Open AI correctly interprets all Gram-positive (49/49) and candida strains (2/2) , but produced 7 mismatches in Gram-negative interpretation (64/71) and could detect both gram species in mixed stains in only one case (1/6) ; COPILOT correctly interpreted 37 Gram-positive strains (37/42), 59 Gram-negative strains (59/71), 1 candida strain (1 /2) and could detect both gram species in 2 cases (2/6) ; GEMINI correctly interpreted 47 Gram-positive strains (47/49), 70 Gram-negative strains (70/71), both candida strains (2/2) and detected both gram mixed species in 3 cases (3/6).

For morphology recognition Open AI correctly interpreted 121 strains (121/129), COPILOT correctly interpreted 114 strains (114/129) and GEMINI correctly interpreted 125 strains (125/129). Overall, Open AI correctly identified without errors for both Gram or morphology 112 strains (112/129), while COPILOT got the correct identification for 92 (92/129) and GEMINI for 119 (119/129).

DISCUSSION

This analysis shows the potential of AI services in the automation and digitization of analytical processes in microbiology with a level of concordance compared to human interpretation reaching at present 90% at best. The use of AI services raises ethical concerns about replacing human labor. However, given the current progresses, AI services in the biomedical field should be considered supplementary but not jet a substitute for the human evaluation.

205 - The future of Parasitology is now: An Effective Molecular diagnostic Protocol for Helminth Diagnosis

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The Future of Parasitology is now: An Effective Molecular diagnostic Protocol for Helminth Diagnosis

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Introduction

The diagnosis of intestinal helminthiasis remains challenging, with microscopy remaining the gold standard procedure despite its time-consuming nature and need for skilled personnel. Molecular techniques, particularly PCR, hold promise for operator-independent diagnostics; however they still face technical obstacles primarily due to insufficient extraction of DNA from parasite ova. In this study, we aimed to establish a molecular diagnostic protocol characterized by a highly effective sample pretreatment designed to disrupt helminth eggs and enhance DNA extraction.

Materials and Methods

A total of 120 fecal samples were collected in 2023 from symptomatic patients presenting with diarrhea and abdominal pain in rural Burundi and dispatched to the Microbiology Unit of University Hospital of Padua in SAF Parapak. Upon arrival, faeces were concentrated and

microscopically inspected. For PCR, aliquots were combined (1:4) with ASL buffer (Quiagen) and 1.8 mm ceramic beads, and then homogenized utilizing the OMNI Bead Ruptor Elite. The samples were then centrifuged followed by total DNA extraction employing magnetic bead extraction technology (Nimbus system). PCR amplification and subsequent interpretation were performed using the Allplex GI-Helminth Assay, a multiplex real-time PCR kit (by Seegene) specifically designed to detect protozoa (*Enterocytozoon spp. / Encephalitozoon spp.)* and helminths *(Strongyloides spp., Hymenolepsis spp., Ascaris spp., Taenia spp., Trichuris trichiura, Ancylostoma spp., Enterobius vermicularis* and *Necator americanus)*

Results

The 120 faecal samples tested with the conventional microscopic techniques revealed 32 samples positive for helminths: 20 for *Ascaris lumbricoides*, 7 for *Trichuris trichiura*, while 5 showed a coinfection of both. Of the samples positive by conventional microscopy, 15 (out of 20) for *Ascaris*, 4 (out of 7) for *Trichuris* and 3 (out of 5) for coinfection resulted to be positive using our extraction procedure plus molecular assay (sensitivity: 84.4% and 75% respectively). Our procedure compared to conventional microscopic techniques yielded 8 false-negative (5 unfertilized *Ascaris* eggs and 3 *Trichuris*) but identified 5 infections missed by microscopy (4 *Trichuris* and 1 *Ascaris*). Sample homogenization proved crucial, as 10 positive samples by microscopy and PCR, if not homogenized yielded false-negative PCR results.

Discussion and Conclusions

The Allplex GI-Helminth Assay paired with the Microlab Nimbus system, demonstrated high sensitivity in detecting fertile *Ascaris* eggs and *Trichuris*. Nevertheless, further standardization of sample collection, storage, and DNA extraction protocol is imperative to enhance accuracy of parasite detection with low fecal loads.

211 - One Tube, Twice the Data: Enabling Integrated Microbiome and Metabolome Profiling from a Single Stool Sample

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One Tube, Twice the Data: Enabling Integrated Microbiome and Metabolome Profiling from a Single Stool Sample

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Introduction:
Large-scale microbiome studies hold great potential for the identification of non-invasive biomarkers for several human diseases. However, the integration of metagenomic and metabolomic workflows often requires multiple sample collection methods, increasing costs and potentially reducing patient compliance in longitudinal protocols. In this study, we evaluated whether two commercially available stool collection devices, eNAT (Copan, Italy) and OMNIgene-Gut (DNA Genotek, Canada), which are designed to preserve nucleid acid for at least 21 days at room temperature, can also reliably stabilize short- and medium-chain fatty acids (SCFAs/MCFAs) in fecal samples.

Materials and Methods:
Thirty-two stool specimens from healthy volunteers were split into untreated (storage in sterile tube without preservative), eNAT-preserved and OMNIgene-Gut-preserved aliquots. After self-sampling, samples were stored at room temperature and analyzed at baseline (t_0) and after 7, 14, and 21 days. Acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic, caprylic, and decanoic acids were quantified by gas chromatography–mass spectrometry (GC-MS) method by subtracting device blanks. Differences at each timepoint were tested by Kruskal–Wallis with Dunn's post hoc; time trends were assessed via linear models (slope ± SE, Benjamini–Hochberg adjustment). Statistical analyses were performed through RStudio 4.4.1.

Results:
At t_0, isovaleric acid in eNAT samples ($p=0.029$) and caprylic acid in OMNIgene-Gut samples ($p=0.009$) differed significantly from untreated stool. Over 21 days, untreated aliquots showed significant increases in all the SCFAs; acetic ($p=0.018$), propionic ($p<0.001$), isobutyric ($p<0.001$), butyric ($p<0.001$) and isovaleric ($p<0.001$) acids. In contrast, none of the nine FAs showed significant changes in either the eNAT or OMNIgene-Gut aliquots over the same period (all $p>0.05$).

Discussion and Conclusions:
Our findings confirm that both eNAT and OMNIgene-Gut devices are fully compatible with fecal fatty acid profiling, allowing for a unified sampling protocol suitable for both nucleic acid

extraction and metabolomic analysis. At baseline, only isovaleric acid (eNAT) and caprylic acid (OMNIgene-Gut) slight differences compared to untreated stool. Thereafter, all measured FAs remain quantitatively stable at room temperature. In contrast, SCFA levels in untreated samples increased over time due to ongoing microbial activity, potentially masking biologically meaningful differences between samples. Adopting a single collection kit cuts per-sample costs, simplifies patient recruitment and logistics, and facilitates the implementation of large-scale, longitudinal microbiome–metabolome studies.

217 - Qualitative molecular rapid assay to detect sexually transmitted infection (STIs): M10 STI panel

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Qualitative molecular rapid assay to detect sexually transmitted infection (STIs): M10 STI panel

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Introduction

Sexually transmitted infections (STIs) are caused by more than 30 different bacteria, viruses and parasites that are known to be transmitted through sexual contact, including vaginal, anal and oral sex. Early diagnosis of STDs is an essential tool for the protection of individual and public health. STANDARD M10 STI Panel is a molecular in vitro diagnostic assay that aids in the simultaneous detection and differentiation of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Human herpesvirus 1* (HSV-1), *Human herpesvirus 2* (HSV-2), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Trichomonas vaginalis* (TV), *Ureaplasma urealyticum* (UU) DNA based on real-time PCR. Panel cartridge contains nucleic acid extraction buffers and real-time PCR reagents for the in vitro qualitative detection.

Materials and Methods

A total of 75 samples (Copan MSwab), derived from vaginal swab having different pathogenic targets, were anonymized and retrospectively collected at the Unit of Microbiology of the Greater Romagna Hub Laboratory (Emilia Romagna Region, Italy) and analysed using STANDARD M10 STI Panel (SD Biosensor, Suwon, Republic of Korea) a novel real-time PCR cartridge-based test completely automated, sample to result. The results obtained in terms of positivity and cycle threshold (Ct) were compared with the reference method Allplex™ STI Essential Assay (Seegene, Seoul, Republic of Korea).

Results

There was almost complete overlap between the two tests, with accuracy values ranging from 98.68% to 100% across all targets. Out of the 75 samples analyzed, only 2 discrepant cases were observed, specifically, in both cases the STANDARD M10 STI panel kit identified the target, while the Allplex STI-7 test did not. The two cases of discrepant target were both from the same sample that was also positive for CT and were characterized by Ct higher than 30. In general, the evaluation of the Ct values for targets identified by both methods revealed no significant differences, confirming the overlap between the two tests.

Discussion and Conclusions

The results of the two tests are almost entirely overlapping, indicating that the STANDARD M10 STI test is highly accurate and reliable in detecting STI-related pathogens. HSV-1 and HSV-2 have not been tested in the panel, meaning that the STANDARD M10 STI Panel has not been tested for identifying these viruses. Further evaluations will be carried out to assess the test's ability to detect these herpesviruses. The speed of execution coupled with the easy to use of STANDARD M10 STI panel make it particularly suitable for decentralized settings (such as clinics, hospitals, or low-resource environments), where quick and simple diagnostics are of outmost relevance.

218 - Optimization of the preanalytical phase in gut microbiota sequencing: study of the impact of fresh face compared with sampling by swab

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Optimization of the preanalytical phase in gut microbiota sequencing: study of the impact of fresh face compared with sampling by swab

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Introduction

There is an accumulating body of evidence that points to a link between a number of diseases and alterations in the gut microbiota, or dysbiosis. This is characterised by a reduction in microbial diversity, the loss of beneficial microbes and the proliferation of harmful bacteria. Faecal microbiota transplantation (FMT) represents an innovative therapeutic approach aimed at restoring gut flora in dysbiotic environments. Following its approval by the FDA in 2013 for the treatment of recurrent *Clostridium difficile* infections, FMT has become a key area of focus within the field of clinical research. The objective of this study is to optimise the process of nucleic acid extraction from faecal samples or swabs, with a view to characterising the microbiota and assessing diversity through sequencing.

Materials and Methods

The study population consisted of 20 subjects with a total of 160 samples. Samples analysed for each subject included non-homogenised fresh faeces, homogenised fresh faeces and swabs, such as FecalSwab™, eNAT® and MSwab®, performed on both homogenised and non-homogenised samples. Samples were processed within 24 hours of collection, otherwise they were excluded. Bacterial DNA was extracted using the QIAamp® PowerFecal® Pro DNA Kit on the QlAcube® Connect MDx platform. Library preparation was performed using the Microbiota Solution B kit (Arrow Diagnostics), which is designed for the V3-V4-V6 hypervariable regions of the 16S rRNA gene. Data analysis was performed using MicrobAT software. To determine which sample provided a more comprehensive characterisation of microbial diversity, the Biodiversity Index, Equitability Index and Shannon Index were evaluated using ANOVA, Kruskal-Wallis and Student's t-tests.

Results

Statistical analysis, using ANOVA tests for the biodiversity index and Kruskal-Wallis tests for Shannon and Equitability, showed that there were no statistically significant differences between the swab and fresh faeces sampling methods on homogenised and unhomogenised starting matrices. The Student's t-test was performed to compare the homogenised and unhomogenised samples from the same sampling method, and there were no statistically significant differences between the two techniques. In addition, the results of the Student's t-test comparing the homogenised and unhomogenised samples regardless of the sampling method were not statistically significant.

Discussion and Conclusions

The statistical analysis performed on the parameters provided by the Microbat software suggest that a representative sample in terms of the selected parameters can be obtained by using fresh faeces, currently considered the gold standard, as well as by using swabs such as FecalSwab™ eNAT® or MSwab® for both homogenised and non-homogenised samples.

222 - Fecal metaproteome changes induced by bariatric surgery in obese patients: a multicenter study

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Fecal metaproteome changes induced by bariatric surgery in obese patients: a multicenter study

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1. Introduction

Bariatric surgery provides effective and durable weight loss in patients with severe obesity. Although many studies have demonstrated the relevant influence of this treatment on the composition of the gut microbiota, little is known about its impact on microbial and host protein profile. To address this need, we aimed to investigate the changes induced in the fecal metaproteome by bariatric surgery.

2. Materials and Methods

A prospective, multicenter, cohort study was conducted in patients with severe obesity, who were randomized between two bariatric surgery techniques (Roux-en-Y gastric bypass and one anastomosis gastric bypass). Fecal samples were collected from 45 obese patients before surgery (T0) and 24 months after surgery (T1). The fecal metaproteome was analyzed by ultra-

high performance liquid chromatography coupled with high-resolution tandem mass spectrometry. An established bioinformatics pipeline was used for peptide and protein identification, quantification, and taxonomic/functional annotation. Statistical analysis of normalized data was performed using a paired-sample t test followed by Benjamini-Hochberg correction for multiple testing.

3. Results

A significant increase in alpha diversity was measured at T1. *Akkermansia*, *Anaerotignum*, *Desulfovibrio*, *Streptococcus*, and *Veillonella* were found to be increased in abundance at T1, while *Faecalibacterium* and *Romboutsia* showed the opposite behavior. The relative abundance of 28 microbial protein functions increased significantly at T1, including enzymes involved in glycolysis, short-chain fatty acid biosynthesis, amino acid metabolism, and cofactor and vitamin biosynthesis, along with several outer membrane proteins encoded by Enterobacterales. On the other hand, a significant decrease in abundance at T1 was observed for other six microbial functions, including proteins involved in oxidative phosphorylation and sporulation. Moreover, the relative abundance of 74 human proteins, belonging to various biological pathways including neutrophil degranulation, increased significantly at T1, whereas other 14 proteins, mainly enzymes secreted by the pancreas and involved in protein digestion, exhibited the opposite trend.

4. Discussion and Conclusions

Our data reveal that bariatric surgery can induce significant changes in the fecal metaproteome, concerning both microbial and human proteins involved in relevant biological functions such as metabolism and immunity. Future studies are needed to further elucidate the molecular aspects of these functional rearrangements and to look for specific associations with follow-up clinical variables.

229 - Rapid microbial diagnosis of sepsis in solid organ transplant (liver) recipients recruited at the University Hospital of Pisa by using a direct-from-blood molecular assay

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Rapid microbial diagnosis of sepsis in solid organ transplant (liver) recipients recruited at the University Hospital of Pisa by using a direct-from-blood molecular assay

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Introduction: Sepsis remains a leading cause of morbidity and mortality in solid organ transplant (SOT) recipients. Due to immunosuppressive therapy, these patients have a blunted immune response and atypical clinical presentations, which complicate early diagnosis. Conventional biomarkers and blood cultures often show reduced reliability, long turnaround times, and limited sensitivity, especially in immunocompromised individuals. This study evaluates a direct-from-blood molecular microbiological assay, which promises to overcome the limitations of conventional diagnostic methods for rapid diagnosis of sepsis.

Materials and Methods: The study, which is conducted within the PNRR Project SEPSOT-CODE, involves SOT (liver) recipients with suspected sepsis enrolled using conventional clinical and laboratory parameters. So far, peripheral blood samples were collected from 10

patients at the onset of suspected sepsis (DS0) and after 1, 3 and 7 days and analyzed by using a direct-from-blood molecular microbiological assay (Bosphore® Sepsis Panel, Anatolia Geneworks), based on multiplex real-time PCR with a turn-around-time of 4-5 h. In parallel, at each time point, conventional blood-culture samples were taken, as well. The Bosphore® Sepsis Panel, identifies 32 pathogens (bacteria and fungi) and 10 resistance genes with a declared sensitivity of 1 CFU/mL for most pathogens analyzed.

Results: The preliminary results obtained demonstrated that 9/10 of patients with suspected sepsis had positive results with Bosphore® Sepsis Panel for early time point samples (DS0/DS1), while corresponding blood cultures were positive for 4 patients. Further tests are currently underway to extend the series of blood samples analyzed.

Discussion and Conclusions: The rapid, direct-from-blood, diagnostic assay utilized seems promising for early detection of bloodstream infections in SOT patients with suspected sepsis. However, testing of higher numbers of samples and further evaluation of later time points (i.e. DS3, DS7), as well as correlation with blood-culture data and/or with other molecular diagnostic methods (e.g. NGS) will be necessary to fully evaluate its diagnostic value.

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237 - Development and Validation of a Metagenomic Analysis Protocol for Cerebrospinal Fluid Samples in Patients with Neurological Cryptogenic Infectious Syndromes

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Introduction

The metagenomic approach by Next Generation Sequencing (mNGS) can serve as a useful method for detecting microbial populations, estimating viral diversity (virome), and complementing classical diagnostics in cases of infectious neurological diseases with unknown origins. In this study we developed and validated an mNGS approach for the identification and characterization of microbial agents in cerebrospinal fluids (CSF) of patients with neurological disorders with suspected infective aetiology. To this aim, we used a pool of CSF samples that resulted negative to routine diagnostic procedures and spiked it with different concentrations of known bacterial and viral agents. Different extraction methods, pre-treatment, and library preparation approaches were tested for finding the best technical conditions in terms of sensitivity and specificity of the method.

Materials and Methods

After nucleic acid extraction, we pre-treated samples by amplifying cDNA by Sequence Independent Single Primer Amplification technique (SISPA) and performing Multiple Displacement Amplification (MDA) technique. NGS libraries were then prepared by fragmentation, adapter ligation and amplification. The sequencing was performed using an Ion-Torrent S5 Prime instrument to obtain 40 million reads per sample. High-quality reads were filtered using Bowtie2 with high stringency parameters to subtract human-derived reads. Filtered reads were then mapped to the NCBI Nucleotide Database with an E-value cut-off of 10-5. All results were merged and grouped by taxonomy using Megan5, collecting the overall numbers of reads mapped to every taxa. Experimental protocol and pipeline were optimized using both samples spiked with known RNA and DNA viruses (i.e., enterovirus and JCV, respectively) and Gram-positive and negative bacterial agents (i.e., Listeria monocytogenes and Haemophilus influenzae, respectively). Green Fluorescein Protein (GFP) was also used as internal control (IC).

Results

No significant differences were observed in the results obtained using the two extraction methods tested (one targeting bacterial DNA and the other targeting viral DNA and RNA). The combined SISPA and MDA approach yields satisfactory results in identifying various pathogens at tested concentrations and detecting IC.

Discussion and Conclusion

mNGS represents a promising approach to support routine diagnostic techniques and a suitable opportunity to investigate the microbiome/virome in the nervous system disorders through an unbiased method able to identify both known and novel pathogens.

239 - Impact of SARS-CoV-2 Pandemic on Neuroinfective Etiology in the Lazio Region

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Impact of SARS-CoV-2 Pandemic on Neuroinfective Etiology in the Lazio Region

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Introduction

The mitigation measures taken to reduce the spread of SARS-CoV-2 have not only suppressed the onset of respiratory infections but have also had an impact on the transmission of other infectious diseases. In this study, we explored the effect of the pandemic on the etiology of central nervous system (CNS) infections by comparing data from cases of suspected neuro-infective syndrome analyzed by using a meningitis/encephalitis molecular syndromic panel, collected in the Lazio region before, during, and after the pandemic.

Materials and Methods

We performed a retrospective analysis of patients with acute meningoencephalitis syndrome who underwent the syndromic FilmArray meningoencephalitis (FA-ME) assay over a period of six years from August 2018 to March 2024 in the Lazio region. The panel simultaneously detects 6 bacteria (S. *pneumoniae*, N. *meningitidis*, S. *agalactiae*, E. *coli K1*, L. *monocytogenes*, H. *influenzae*), 7 viruses (HSV-1, HSV-2, VZV, EV, Human Parechovirus, HHV6 and CMV) and 2 fungi (Cryptococcus *gatii/neoformans*). According to the collection data, the FA-ME results of 4,203 cerebrospinal fluids (CSF) were split into three groups: pre-pandemic (August 2018-February 2020; n. CSF:1,050), pandemic (March 2020-March 2022; n. CSF:1,253) and post-pandemic (April 2022-March 2024; n. CSF:1,900) respectively. The CSF positivity of each pathogen was assessed individually and compared among the three groups. Statistical analysis was performed using GraphPad Prism (8.0.1). A p value < 0.05 was considered statistically significant.

Results

Pathogens were identified in 193/1,050 (18%), 132/1,253 (10%) and 215/1,900 (11%) of pre-pandemic, pandemic and post-pandemic groups, respectively. Results showed that viral pathogens were the most frequently detected in all groups, although the S. *pneumoniae* was the most representative pathogen. Comparing pre-pandemic to pandemic, statistical analysis showed a significant reduction in neuro-infections caused by EVs ($p<0.0001$), S. *pneumoniae* ($p<0.001$) and N. *meningitidis* ($p=0.049$) while the other pathogens were not affected. EVs cases showed a drastic decline during the pandemic, ranging from a frequency of 3.52% to 0.08%. However, even though EVs cases increased to 1.32% in the post-pandemic period, they remained significantly lower than in the pre-pandemic period.

Conclusion

During the pandemic, the epidemiology of pathogens causing neurological infections has changed, resulting in a significant reduction in CNS infections by airborne pathogens, both viral and bacterial, as expected given the countermeasures taken to contain the spread of SARS-CoV-2. Although cases of S. *pneumoniae* and N. *meningitidis* have returned to pre-pandemic values after the pandemic, the prevalence of EVs cases appears to be significantly lower than in the pre-pandemic period.

241 - CT matters: a comparative study of two PCR assays for Candida auris surveillance

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CT matters: a comparative study of two PCR assays for Candida auris surveillance

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Introduction

Candida auris (*C. auris*) is an emerging multidrug-resistant yeast causing invasive disease with high mortality. Due to its ability to survive in the environment and efficiently disseminate within immune-compromised patients in a healthcare setting, early identification of colonization is important for its prevention and control. In response to the growing threat, the Italian Ministry of Health has released a National Protocol that provides guidance for the identification, surveillance, reporting and control of *C. auris* infections and colonization. Early diagnosis, especially in high-risk patients, is the cornerstone of control and containment. There is no consensus in the literature regarding the superiority of culture-based versus molecular-based methods for screening, but the epidemiology of *C. auris*, which has a very rapid spread, supports the possibility of a better performance of molecular tests. Such tests are more sensitive, have faster detection times and can be powerful tools for rapidly identifying carriers and enacting successful infection control measures.

Materials and Methods

Simplexa®*C. auris* Direct Kit (Diasorin), is a CE-IVDR marked real-time PCR assay for the qualitative detection of *C. auris* DNA without extraction from composite swabs of axillas and groin. The assay is run on the LIAISON® MDX platform. AurisID (Olm Diagnostics) is a CE IVD-labelled real-time PCR test for the identification of *C. auris* from swabs (axillary/ inguinal), serum and plasma. Both methods require minimal sample preparation and deliver results in less than two hours.

Results

In this preliminary study, we compared the performance of two commercially available real-time PCR assays for the detection of *C. auris*. Different swabs (axillary and inguinal) from a single patient were analysed using the Simplexa®*C. auris* Direct Kit and AurisID. The comparison of CT values demonstrated an average difference of 6 to 12 CT, where Simplexa®*C. auris* Direct Kit exhibited lower CT values. No false positives were observed in negative control swabs for either assay. Workflow analysis highlighted fewer manual steps and no DNA extraction for Simplexa®, while AurisID required manual PCR setup.

Discussion and Conclusions

Real-time PCR is a crucial tool for the rapid and sensitive detection of *C. auris*, and different approaches are being used. Our preliminary data suggest that the Simplexa®*C. auris* Direct shows higher performance in terms of CT values and offers a more streamlined and automated workflow compared to AurisID. These features make it well suited for active surveillance settings, where rapid turnaround and standardized procedures are critical. Further studies on a larger sample size are needed to confirm diagnostics performances and reproducibility of both methods.

250 - Viral load monitoring in cervical and self-collected vaginal samples from women with persistent Human Papillomavirus 16 (HPV16) infection

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Viral load monitoring in cervical and self-collected vaginal samples from women with persistent Human Papillomavirus 16 (HPV16) infection

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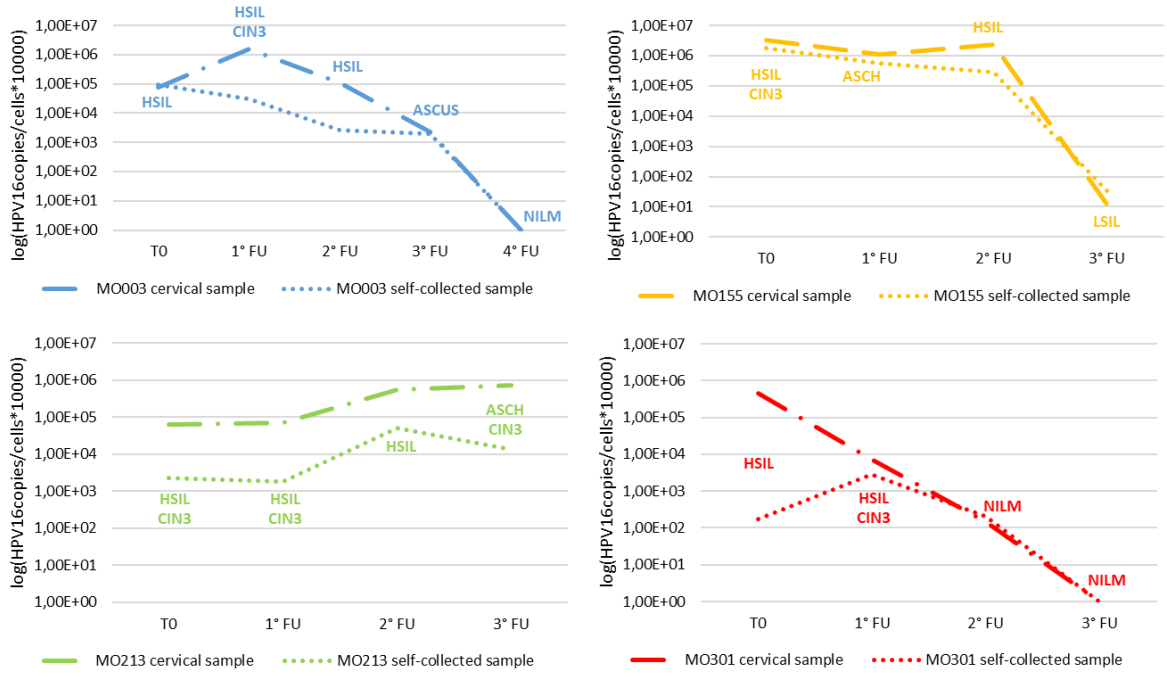
Introduction: The introduction of Human Papillomavirus (HPV) testing into cervical cancer screening programs has enabled the use of vaginal self-collection as an alternative to clinician-collected samples. Persistent HPV infection may lead to precancerous or cancerous lesions, with HPV16 being the most frequently detected genotype in women with high-grade dysplasia. However, cytology triage of HPV-positive women cannot be performed on vaginal samples, and alternative novel molecular biomarkers are under investigation. This study investigates the potential role of viral load as a biomarker of disease progression in women with persistent HPV16 infection, using both clinician-collected and self-collected vaginal samples.
Materials and Methods: 358 women were referred to the Colposcopy Clinic of IRCSS San Gerardo dei Tintori (Monza, Italy) for a previous abnormal cervical cytology (T0) and followed according to the local clinical protocol. To date, 129 women returned for the first, 44 for the second, 11 for the third, and 2 for the fourth follow-up (FU) visits. Clinician-collected cervical and vaginal self-collected samples were obtained at each time point and HPV-typed with Anyplex™II HRHPV (Seegene). In case of HPV16 persistent infection for at least two consecutive time points, samples were also tested using OncoPredict HPV Quantitative Typing assay (Hiantis) to determine viral load, expressed as (HPV copies/n°cells)*10,000.
Results: At enrolment HPV positivity rates were 66.2% (239/358) and 71.2% (255/358) in cervical and vaginal samples, respectively. Among women returning at subsequent FU visits, positivity rates in the two sample types were respectively 45.7% (59/129) and 54.3% (70/129) at the first, 34.1% (15/44) and 45.4% (20/44) at the second, 45.4% (5/11) and 63.6% (7/11) at

the third and 50.0% (1/2) and 50.0% (1/2) at the fourth visit. HPV16 was the most frequently detected genotype at each time point in both specimen types. In particular, HPV16 persistent infections were detected in 15 cervical samples at the first, 5 at the second, and 4 at the third FU visits. Trends in viral load in the two sample types were similar, although with different absolute values; overall viral loads correlated with the progression/regression of the cervical lesions (Figure 1).

Discussion and Conclusions: These preliminary findings confirm that HPV16 are frequently associated with persistent infections and suggest that viral load in cervical and vaginal specimens may be useful for disease monitoring. Further studies are needed to validate the clinical utility of viral load assessment in the management of women with cervical abnormalities.

Figure 1: Evolution of viral load in HPV16 persistent infection at 4 time points in cervical and vaginal specimens.



257 - Digital PCR for identifying inherited chromosomally integrated HHV-6 in transplant recipients

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Digital PCR for identifying inherited chromosomally integrated HHV-6 in transplant recipients

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Introduction. This study aimed to investigate human herpesvirus 6 (HHV-6) infection in patients admitted to the Sant'Orsola Polyclinic and to evaluate the utility of advanced diagnostic techniques, specifically digital PCR (dPCR), in resolving diagnostically challenging cases, particularly in transplant recipients, when conventional methods might not be completely exhaustive.

Materials and Methods. HHV-6 DNA was detected in blood, hair follicles (HF) and paraffin-embedded biopsies using the HHV-6 ELITe MGB® kit (ELITechGroup, Italy). HHV-6A/B discrimination was performed with the AltoStar® HHV-6 PCR Kit 1.5 (Altona, Germany). dPCR assay targeting HHV-6 and the housekeeping gene RPP30 was performed on the QIAcuity® platform (QIAGEN, Germany). The HHV-6 copies number per cell was calculated using the formula HHV-6/(RPP30/2), based on the assumption of two copies of RPP30 per diploid genome. A ratio ≥1 was considered consistent with inherited chromosomally integrated HHV-6 (iciHHV-6).

Results. Between January 2012 and April 2025, iciHHV-6 was diagnosed in 76 of 3046 patients (2.5%) investigated for HHV-6. Among 52 transplant recipients with HHV-6 load >3 Log_{10} copies/mL, three required additional testing beyond conventional HHV-6 PCR on blood and HF. Two were allogeneic hematopoietic stem cell transplant (HSCT) recipients who had low HHV-6A and HHV-6B levels in blood, respectively, but tested positive for HHV-6 in HF. dPCR in both cases showed HHV-6 DNA/cellular DNA ratio <<1 in blood, ruling out iciHHV-6 in the graft. The third patient was a liver transplant (LT) recipient with HHV-6B detected in blood (6.5 Log_{10} copies/mL) and HF and a relatively low viral load (3.0 Log_{10} copies/10 ng) in liver biopsy. dPCR showed a ratio >1 in blood but <<1 in the biopsy, ruling out iciHHV-6 in the graft.

Another case requiring additional testing involved a LT recipient with 4.5 Log_{10} copies/mL HHV-6A in blood and 4.0 Log_{10} copies/10 ng in liver biopsy, but negative HHV-6 PCR in HF. dPCR revealed a ratio <<1 in blood and >1 in the biopsy, suggesting iciHHV-6 in the graft.

Discussion and Conclusions. Transplantation carries the risk of transmitting iciHHV-6 to recipients. In HSCT, the recipient's blood is repopulated with donor-derived cells, making diagnosis with traditional methods challenging. In addition, the release of chromosomal DNA from non-hematopoietic cells may occur, potentially complicating the interpretation of HHV-6 DNA levels in blood. In these scenarios, dPCR has proven to be a critical tool, providing absolute HHV-6 DNA quantification, and helping to avoid unnecessary and potentially harmful antiviral treatment.

265 - Innovative strategies of fast microbiology for the early diagnosis of sepsis

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Innovative strategies of fast microbiology for the early diagnosis of sepsis

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Introduction: Bloodstream infections (BSI) represent a clinical challenge of considerable complexity and high mortality thanks to their capacity to trigger a systemic inflammatory response and lead to sepsis . Timely diagnosis of these infections, traditionally reliant on blood culture, is crucial for guiding effective therapeutic intervention. However, classical microbiological methods, while remaining the gold standard, often encounter time constraints that can delay the initiation of targeted therapy. This study evaluated the impact of innovative "fast microbiology" strategies on the speed and accuracy of sepsis diagnosis, with particular attention to the UNIO A24S (Anatolia geneworks) instrument for rapid PCR.

Materials and Methods: The study included a comparative analysis of whole blood samples (blood count) from all suspected sepsis patients. 86% of the samples were processed with both the standard method and Real-Time PCR technology using the UNIO A24S instrument. UNIO A24S is an automated system for the extraction and amplification of nucleic acids that identifies the main bacteria responsible for sepsis and their related resistance genes in 6 hours. We also analyzed the role of biomarkers, including Procalcitonin (PCT), C-reactive protein (PCR), and Adrenomedullin (ADM), as complementary tools for the early diagnosis and monitoring of sepsis.

Results: The results obtained highlight how "fast microbiology" techniques can effectively accelerate reporting times, optimize antibiotic therapy, and improve patient prognosis. UNIO A24S enabled a significant reduction in reporting time compared to traditional blood culture, allowing for earlier identification of pathogens and their resistances. The concordance between UNIO A24S results and blood culture was evaluated, highlighting the advantages and limitations of each method in terms of time and sensitivity.

Discussion and Conclusions: In conclusion, the integration of innovative “fast microbiology” strategies represents a promising opportunity for improving the clinical management of sepsis. In particular, the use of rapid PCR systems such as UNIO A24S represents a significant advancement in the early diagnosis of sepsis. The ability to obtain results in a short time can improve the clinical management of patients, optimize the use of antibiotics, and potentially reduce mortality. However, further studies are necessary to define optimal protocols for the use of these technologies and to evaluate their long-term impact on clinical outcomes and the spread of antimicrobial resistance.

270 - Quantitative molecular assay to diagnose HBV from plasma with Anatolia Geneworks®

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Quantitative molecular assay to diagnose HBV from plasma with Anatolia Geneworks®

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Introduction

Hepatitis B virus (HBV) is a small DNA virus belonging to the Hepadnaviridae family. With more than 2 billion people showing serologic evidence of past or current infections and approximately 257 million chronically infected individuals, HBV infection is a major global health problem and is known to cause a variety of liver diseases, including acute hepatitis, chronic hepatitis, cirrhosis, and liver cancer. The aim of the study was to evaluate and compare the results obtained using the Bosphore HBV Quantification Kit (Anatolia Geneworks, Istanbul, Turkey) with those obtained using a routine reference Aptima® HBV Quant Assay (Hologic Inc., San Diego, United States). The clinical sensitivity and specificity of the Bosphore HBV Quantification Kit were also evaluated.

Materials and Methods

A total of 130 plasma samples were anonymized and retrospectively collected at the Microbiology Unit of Greater Romagna Hub Laboratory (Region Emilia Romagna, Italy). Nucleic acid extraction was performed by UNIO B24 Extraction System (Anatolia Geneworks, Istanbul, Turkiye), using the extraction kit UNIO Viral DNA-RNA Extraction Kit 600µl with 600µl starting and 60µl elution volume, both provided by Anatolia Geneworks. The Real-Time PCR was performed by Montania 4896, employing the Bosphore HBV Quantification Kit, an in vitro diagnostic Real-Time Polymerase Chain Reaction (qPCR) kit intended for quantitative detection of HBV viral DNA.

Results

A total of 130 plasma samples were analyzed, and no invalid results were observed. Only one sample (0.77%) showed a discrepant result, testing low positive with the Bosphore HBV Quantification Kit and negative with the Aptima® HBV Quant Assay. The overall concordance rate between the two methods was 99.23%. Among the concordant positive results, 109 out of 110 (99.1%) exhibited viral load values within ±0.5 $\log_{10}$ IU/mL between the two assays. The Bosphore HBV Quantification Kit demonstrated 100% sensitivity and 95.0% specificity.

Discussion and Conclusions

The Bosphore HBV Quantification Kit demonstrated a high concordance rate of 99.23% when compared to the reference method utilized in this study. Notably, there was one discordant result observed, in which the Bosphore assay detected HBV DNA whereas detected as negative with the Aptima® HBV Quant Assay. This observation could reflect higher analytical sensitivity of the Bosphore kit to potentially identify very low viral loads that may be missed with some alternative methods. The results affirm the validity and clinical utility of the Bosphore HBV Quantification Kit to sensitively and precisely identify HBV DNA in plasma samples.

275 - Characterization of rhinovirus-positive bronchiolitis cases during six epidemic seasons in Rome.

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Characterization of rhinovirus-positive bronchiolitis cases during six epidemic seasons in Rome.

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Introduction. Rhinoviruses (HRVs) are a leading cause of respiratory infections and a wide range of diseases in both children and adults. Despite their etiological role, the disease burden of HRVs in cases of bronchiolitis is still not well understood. Additionally, the distinguishing characteristics of the three HRV species, if any, remain unclear. Therefore, this study aimed to characterize HRV-positive bronchiolitis cases clinically and virologically. The study was conducted at a pediatric hospital in Rome during six epidemic seasons, both before and after the pandemic period.

Materials and Methods. Infants who attended the Sapienza University Hospital in Rome during the epidemic seasons (October–April) from 2016–2017 to 2019–2020 and then in October–April of 2023–2024 and 2024–2025 were enrolled if they had a clinical diagnosis of bronchiolitis, no underlying chronic diseases, and were not premature. Informed consent was obtained from parents. Demographic and clinical data were obtained from medical files. Nasopharyngeal washings were tested for 14 respiratory viruses using molecular methods. Genotyping was performed via Sanger sequencing of the 5' UTR tract.

Results. A total of 138 bronchiolitis cases positive for HRV were enrolled during the study period. The HRV positivity rate ranged from 13% to 25% of the total bronchiolitis cases in each

season. The rate of HRV co-infection with other respiratory pathogens fluctuated by epidemic season, ranging from 30% to over 60%. More than half of the cases (n = 76) were successfully genotyped by sequencing. HRV-A was the most prevalent species (40/76, 53%), followed by HRV-C (27/76, 35%), and then HRV-B (9/76, 12%). No respiratory enterovirus was detected among the genotyped bronchiolitis cases. Infants positive for HRV-B were more frequently coinfected with other respiratory viruses ($p < .001$ and exhibited greater clinical severity due to coinfections ($p < .001$). Comparing demographic and clinical data between patients monoinfected with HRV-A (n = 32) and HRV-C (n = 18) revealed that the HRV-C group had slightly higher clinical severity scores ($p = .055$). Bronchiolitis was caused by a significant number of different genotypes: HRV-A (N = 22) and HRV-C (N = 13). The only genotype associated with more than three cases was HRV-A 78 (N = 5).

Discussion and Conclusions. HRVs play a significant role in infant hospitalization due to bronchiolitis. Each epidemic season, HRVs presented with a variety of genotypes, predominantly the HRV-A species. As nirsevimab will reduce the burden of RSV bronchiolitis, further research is needed to characterize the clinical burden of HRV bronchiolitis and the molecular epidemiology of the related strains.

282 - Using machine learning for predicting antibiotic resistance in bloodstream infections

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Using machine learning for predicting antibiotic resistance in bloodstream infections

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Antibiotic resistance poses a significant challenge in modern medicine, with current tests requiring 24-48 hours to determine susceptibility. In critical cases, this delay can be life-threatening. Therefore, a rapid and accurate method for predicting antibiotic resistance is essential. This purpose of this study is the development and comparison of several machine learning/deep learning models to predict antibiotic resistance or susceptibility starting from species identification using epidemiological data. While most studies focus on urinary tract infections or all infections, due to the availability of large datasets, few address specifically bloodstream infections despite their clinical importance.

Models were trained using hospital data acquired from the Bologna metropolitan area, for patients with positive blood cultures between January 2024 and December 2024. The following pathogen-antibiotic combinations were considered for this study: *S.aureum*-Oxacillin, *E.faecium*-Vancomycin, *E.coli*-Cefotaxime, Ceftazidime and *K.pneumoniae*-Cefotaxime, Meropenem. Input features include patients demographics (age, sex), time the blood culture was taken, identified species, antibiotic used and antimicrobial resistance (AMR) rates in the different hospitals. Models were built using Python and the sklearn and PyTorch libraries. The employed models were logistic regression, random forest, XGBoost and Multi Layer Perceptron.

The predictive value of the machine learning models is modest, with values for the area under receiver operating characteristic curve (ROC-AUC) ranging from 0.59 to 0.84, depending on the pathogen-antibiotic combination used. These values are similar to previous results found in literature.

Important predictive features such as historical antibiotic exposure and patient medical records were unavailable in the current dataset. Future iterations will incorporate these and

other clinically relevant variables to optimize model performance and enhance predictive precision.

290 - FAM19A4 and mir124-2 methylation in oral HPV-related diseases

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FAM19A4 and mir124-2 methylation in oral HPV-related diseases

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INTRODUCTION. Persistent Human papillomavirus (HPV) infection has been implicated as a major cause of the rising incidence of oropharyngeal and oral cancers. These diseases are characterised by delayed diagnosis, and even when identified effectively, the prognosis of precancerous lesions is often difficult to understand. The search for new epigenetic biomarkers is an appropriate way to identify neoplastic and preneoplastic lesions and to characterise

cancer progression. Among the assessable epigenetic mechanisms, DNA methylation has proven to be one of the most suitable.

MATERIALS AND METHODS. 111 oral rinses were analysed for the DNA methylation of two onco-suppressor genes, FAM19A4 and mir124-2. According to histological diagnoses, samples were divided into three groups: benign lesions (BL), potentially malignant disorders (OPMD) and oral cancers (OSCC). A fourth group of patients without lesions (NL) was added. INNO-LIPA HPV Genotyping Extra II kit (Fujirebio, Japan) was used to detect HPV, while PreCursor-M+ kit (Fujirebio, Japan) allowed the evaluation of hypermethylation based on real-time PCR. The value of ΔCt was then used to evaluate the methylation level using the ΔΔCt method, which is based on the application of the following formula: ΔΔCt=ΔCtsample-ΔCtcalibrator. The ΔΔCt ratio formula ($2^{-\Delta\Delta Ct}$) was used to express the ΔΔCt figure as '% of methylated cells in the sample'.

RESULTS. Hypermethylation levels correlated with the severity of oral lesions for both targets individually. Statistical analysis of the median ΔΔCt values of the two targets confirmed this observation. *FAM19A4* scores were significantly correlated with the diagnosis, with OSCC score significantly higher than NL (0.28vs0.0, $p<0.05$), BL (0.28vs0.0, $p<0.05$), and OPMD (0.28vs0.03, $p<0.05$). *Mir124-2* $2^{-\Delta\Delta Ct}$ values analysis showed similar results: OSCC score was greater than NL (048vs0.14, $p<0.05$), and BL (048vs0.09, $p<0.05$). HPV16-positive OSCCs were selected to evaluate whether their methylation levels were higher than other HPV-positive samples: HPV16 was associated with the hypermethylation of both targets.

DISCUSSION AND CONCLUSIONS. This study showed that both targets are hypermethylated in oral cancers, and that the methylation level increases progressively from patients without lesions to OPMD and OSCC. The methylation status of *FAM19A4* and *mir-124-2* may play an important role in oral cancer progression and, hence, in defying the prognosis of OPMD patients, whose hypermethylation would suggest a close follow-up. Moreover, HPV16's association with hypermethylation is a further suggestion of its actual involvement in oral carcinogenesis. To confirm these results and gain further insight into the role of HPV in methylation impairment, the sample size will be expanded.

291 - A Pilot Study on Prevalence of Human Papillomavirus in Partners of Women Attending Cervical Cancer Screening: focus on potential impact of the vaccination

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A Pilot Study on Prevalence of Human Papillomavirus in Partners of Women Attending Cervical Cancer Screening: focus on potential impact of the vaccination

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1. **Introduction**

Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STIs), with similar global prevalence rates in men and women. The focus of most studies to date has been on HPV infection in women, as it is the cause of over 90% of cervical cancers. However, the asymptomatic nature of male HPV infection, the lack of screening programs for men, and their role as reservoirs may influence the likelihood of transmission to female partners and, thus, the risk of developing cervical lesions. In light of the above, we aim to assess HPV prevalence in 90 male partners of HPV-infected women, focusing on the impact of male HPV infection on female partners' cervical health and the potential influence of HPV vaccines.

2. **Materials and Methods**

For each patient were collected two samples, genital and urethral swabs. HPV detection and genotyping were performed by Allplex HPV28 detection assay (Seegene).

3. **Results**

HPV-DNA was detected in 66.7% (60/90) of genital samples. High-risk HPV (hrHPV) genotypes were detected in 90% (54/60) cases, and multiple infections in 55% (33/60). The most frequent hrHPVs were HPV31 (p=0.0265) and HPV52 (p=0.002), found in 18.3% (11/60) of cases, and HPV53 (p=0.0116) in 16.7% (10/60). A higher probability of a less severe cytological diagnosis with the increase in the number of genotypes detected (p =0.0146) was shown from statistical analysis. The HPV-positive partners of females with cervical lesions, had vaccine genotypes of the quadrivalent and nonavalent vaccines in 18.7% (6/32) and 62.5% (20/32), respectively. A significantly higher potential to prevent lesions (62.5% vs. 18.7%, p = 0.0001) was showed by the nonavalent vaccine, with an absolute additional potential impact (AAI) of 31.1% in histological diagnoses.

4. Discussion and Conclusions

Of the most common and significant genotypes found in our study population, only HPV31 and HPV52 are included in current vaccine formulations, but HPV53 and HPV42 have also been described in the male population by Buttà et al. Other studies of male partners of HPV-positive women have found HPV16 as the most common genotype. However, given the limited literature on this population, we can hypothesize a change in circulating genotypes following the vaccination of female cohorts, which is in line with the “type replacement” phenomenon described by Chen et al. From statistical analysis a significantly higher potential to prevent lesions (p = 0.0001) was showed by the nonavalent vaccine. This pilot study correlates the number of partner genotypes with the severity of cervical lesions, elucidates the genotypes distribution in this population, and shows promising results on the preventive potential of vaccinating male partners.

292 - Comparing Urine and Urethral Swabs Testing for Human papillomavirus Diagnosis in Men: A Non-Invasive Approach

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Comparing Urine and Urethral Swabs Testing for Human papillomavirus Diagnosis in Men: A Non-Invasive Approach

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1. Introduction

The understanding of Human papillomavirus (HPV) infection in males remains limited, with significant knowledge gaps regarding optimal specimen selection for accurate diagnosis. Many studies have suggested the combination of genital and urethral swabs as the most effective sampling approach, but the latter can be less acceptable to patients. Consequently, our study aimed to evaluate whether a urine sample combined with a genital swab could serve as a suitable alternative to a urethral swab for an effective diagnosis of HPV infection in male patients.

2. Materials and Methods

For each of the 74 recruited patients, three distinct sample types were collected: a genital swab, a urethral swab, and a urine sample that underwent appropriate concentration. HPV-DNA detection was performed using the INNOLiPA® HPV Genotyping Extra II assay (Fujirebio, Tokyo, Japan).

3. Results

The prevalence of HPV infection in the male cohort was 74.3% (55/74), with high-risk HPV (hrHPV) genotypes detected in 85.4% (47/55) of these cases. Multiple HPV infections were observed in 56.4% (31/55) of the HPV-positive individuals. Comparative analysis of the three sample types revealed concordance (all three samples positive or negative) in 51.4% (38/74) of the participants, while 48.6% (36/74) showed discordant results (only one or two samples positive). Specifically, the genital swab yielded the highest detection rate of HPV infection at 91% (50/55), followed by the urethral swab at 60% (33/55) and the urine sample at 50% (28/55). Notably, among the 9% (5/55) of genital swabs that tested negative for HPV-DNA, 60% (3/5) of the corresponding urethral swabs and 80% (4/5) of the urine samples were positive. The combination of genital and urethral swabs increased the detection rate of HPV infection to 96.4% (53/55), while the combination of the genital swab and urine sample allowed for the detection of 98.2% (54/55) of HPV infections.

4. Discussion and Conclusions

Recent studies in the literature have suggested urine-based sampling as suitable specimen for HPV DNA detection in women. Conversely, research focusing on male population are limited, often concentrated on subjects with risky behavior and presents inconsistent findings regarding urine samples. These discrepancies may arise from the use of random urine collections rather than first-void urine, and inadequate sample processing. The data from the present study suggest that the combination of a genital swab and urine may represent an effective approach for diagnosing HPV infection in men. Urine sampling appears to be a promising alternative to urethral swabs, potentially enhancing patient compliance due to its non-invasive nature. Larger studies should assess adding urine tests to routine male HPV diagnosis.

301 - Membrane Vesicles of Bacterial Species Associated with Dysbiosis in the Development of Atrial Fibrillation

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Membrane Vesicles of Bacterial Species Associated with Dysbiosis in the Development of Atrial Fibrillation

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Introduction: A correlation between intestinal dysbiosis and atrial fibrillation has been recently demonstrated with a hyperproliferation of Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*), as well as an increased systemic LPS concentration. Bacterial Membrane Vesicles (MVs) are spherical lipid structures which affect organs and tissues far from the primary infection site via the blood system. We speculate the potential role of MVs released by the abovementioned species related to gut dysbiosis, in the development of atrial fibrillation.
Materials and methods: *E. coli* and *K. pneumoniae* MVs, isolated via ultracentrifugation, were characterized via Dynamic Light Scattering (DLS), Nanotracking Analysis (NTA), Transmission Electron Microscopy (TEM) and proteomic analysis by using LC-MS/MS system. The characterized MVs were then administered to human macrophages under basal and

proinflammatory conditions (LPS stimulus) as well as to human primary atrial fibroblasts to determine their biological and immunomodulatory effects.

Results: TEM analysis showed intact spherical structures ranging in diameter from 50 to 500 nm for both bacteria. DLS analysis showed two vesicular populations of *E. coli* MVs, having hydrodynamic diameter of 141-23.69 nm. *K. pneumoniae* MVs showed no clearly distinct populations, with hydrodynamic diameter within the range of 20-109.3 nm. NTA analysis displayed a concentration of 9.42×10^{12} particles/mL for *E. coli* and a concentration of 2.18×10^{11} particles/mL for *K. pneumoniae*. MVs from both bacteria have immunostimulatory effect on macrophages at high concentrations under basal conditions comparable to LPS. Under pro-inflammatory conditions, both MVs showed additive effects compared with LPS at high concentrations but display no effect at low concentration. MVs administration to atrial fibroblasts shows cytotoxicity. Molecular analysis is ongoing to identify potential mechanism of action on both the cell types.

Discussion and conclusions: The data showed that MVs of *E. coli* and *K. pneumoniae* have distinct physicochemical characteristics as well as different populations and are capable of stimulating macrophages, which represent the frontline cells in inflammatory processes. Biological effects from cells of the atrial compartment are confirmed. These findings, together with ongoing proteomic analysis, represent the first step to investigate the potential involvement of bacterial vesicles in atrial fibrillation and their use as new biomarkers for early diagnosis.

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309 - Advanced Crystal Digital PCR-Based Approach for West Nile Virus detection: from genome identification to lineages typing.

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Advanced Crystal Digital PCR-Based Approach for West Nile Virus detection: from genome identification to lineages typing.

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Introduction: West Nile Virus (WNV) is an arbovirus transmitted by mosquitoes of the *Culex* genus, which can cause clinical manifestations ranging from mild febrile states to severe encephalitis. Currently, human WNV infections are caused by lineages 1 and 2, and the risk of developing encephalitis is more closely related to lineage 1 infections. The development of a fast and effective diagnostic test for WNV infection and lineage identifications is critical for a rapid choice of proper treatment. In recent years, Cristal Digital PCR (cdPCR) has proven to be highly effective and sensitive for the detection of pathogens based on a microfluidic droplet system. The aim of this study is to develop a WNV detection protocol based on cdPCR.
Materials and Methods: Viral RNAs were extracted from arthropods and avian organ samples and were retrotranscribed.To set up the cdPCR assay, we constructed two plasmids containing the amplification products obtained with the primer pair use in cdPCR assay. Partition, amplification and reading were performed on the Prism6 Naica System (Stilla Technologies) using 10X Naica™ Multiplex PCR MIX. To discriminate lineage 1 form Lineage 2 we used two different probes.
Results: Primary outcomes revealed that this protocol was effective to detect WNV infections in clinical samples. In addition, the use of two different probes allowed discrimination between WNV-1 and WNV-2. We have also tested the effect of the spectral overlap for the fluorophores for the two lineages and evaluated the limit of detection (LOD).
Conclusions: The proposed protocol proves to be effective for the detection of WNV infection. Moreover, it allows to clearly distinguish between the two lineages using different probes.

cdPCR allows absolute quantification and higher sensitivity in target detection than Real Time-PCR. The ability to rapidly and accurately identify WNV makes this method a promising tool for rapid diagnostic, epidemiological surveillance and timely response to potential outbreaks.

316 - Detection of pathogenic fungi causing geotrichosis: development of a surveillance assay

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Detection of pathogenic fungi causing geotrichosis: development of a surveillance assay

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Aims and Hypothesis: *Magnusiomyces clavatus*, *M. capitatus*, and *Geotrichum candidum* are emerging opportunistic fungi that are phylogenetically related and often misidentified. These yeast-like organisms are responsible for severe respiratory infections, collectively referred to as geotrichosis, primarily affecting immunocompromised patients. Current diagnostic approaches, such as plate culture followed by MALDI-TOF Mass Spectrometry, are time-consuming and not suitable for rapid or high-throughput screening. The aim of this study is to develop and validate a real-time PCR (qPCR) assay for the rapid detection of these pathogens in respiratory samples, specifically bronchial aspirate (BASP), bronchoalveolar lavage (BAL), and sputum collected from immunocompromised individuals. Additionally, we aim to set up a multiplex qPCR assay capable of differentiating the three species.

Scientific Rationale: Timely and accurate identification of fungal pathogens in respiratory specimens is essential to guide appropriate antifungal therapy and improve clinical outcomes. *M. clavatus* and *M. capitatus* are intrinsically resistant to echinocandins, a class of antifungals administered as prophylaxis therapy, particularly caspofungin, for hematological patients. Given the high mortality rates associated with these infections, a prompt change in antimycotic therapy would be of paramount importance.

Preliminary Data: *M. clavatus*, *M. capitatus*, and *G. candidum* mitochondrial DNA was used as the molecular target for qPCR primer design. Despite the phylogenetic closeness of these 3 fungi, a comparative analysis of their mitochondrial genomes revealed sufficient sequence divergence to enable species-specific primers to be designed. Both pan-Geotrichum-like and species-specific mitochondrial primers were designed and tested, showing promising preliminary results in terms of specificity and amplification efficiency.

Future Perspectives: The epidemiology and ecological niche of these emerging fungi remain poorly defined. In particular, their natural reservoirs and the potential existence of asymptomatic human carriers are still unexplored. The development of qPCR assays could be employed as valuable tools for future studies aimed at elucidating transmission dynamics, environmental sources, and colonization patterns of these emerging pathogens.

318 - Evaluation of a Fully Automated System for Rapid AST Directly from Positive Blood Cultures in Gram-Negative Infections: Performance and Impact on Time-to-Result

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Introduction. The increasing prevalence of antimicrobial resistance poses a major global health challenge, particularly in the management of bloodstream infections (BSIs) caused by Gram-negative bacteria. Rapid identification of effective antibiotic therapy is crucial to improving patient outcomes and limiting the spread of resistance. The aim of the study was to assess the performance and impact on time to result of ASTar® (Q-linea), a fully automated platform for rapid antimicrobial susceptibility testing (AST) directly from positive blood cultures, with results in approximately six hours.

Materials and Methods. The performance of ASTar was evaluated in comparison to the reference broth microdilution method (Sensititre, Thermo Fisher Scientific) for 13 antibiotics, and to a gradient strip test (MTS™, Liofilchem) for 2 antibiotics (ceftazidime-avibactam and ceftolozane-tazobactam). The MICs obtained were interpreted according to EUCAST breakpoints (v.15.0). A total of 36 prospective positive blood culture (BC) samples from hospitalized patients with Gram-negative bacterial infections were analyzed. To further evaluate the system's performance across a range of resistant phenotypes, 8 negative BC bottles were contrived with 8 well-characterized, multidrug-resistant Gram-negative strains. Time-to result was also compared.

Results. Among the 44 samples analyzed, 33 were Enterobacterales, including *Klebsiella pneumoniae* (n=14), *Escherichia coli* (n=11), *Klebsiella aerogenes* (n=1), *Klebsiella oxytoca* (n=2), *Serratia marcenscens* (n=1) and *Proteus mirabilis* (n=4). Other pathogens identified included *Pseudomonas aeruginosa* (n=9), *Acinetobacter baumannii* (n=1), and *Haemophilus*

influenzae (n=1). ASTar demonstrated an overall Categorical Agreement (CA) of 94.2% and Essential Agreement (EA) of 93,3%. Cefotaxime, aminoglicosides, levofloxacin, tigecycline and colistin reached a 100% CA and EA. On the other hand, cephalosporines other than cefotaxime, alone or with beta-lactam inhibitors, showed EA between 76% (cefepime) and 90%, and CA between 79% (ceftazidime-avibactam) and 92%. MICs from ASTar were generally higher than MICs from Sensititre, mostly by 2 two-fold dilution when EA was not reached. Overall, the percentages of minor errors, major errors and very major errors were 3.6%, 3.4% and 0%, respectively. On average, ASTar results were available 32 hours earlier than those obtained with the standard method.

Discussion. ASTar provides rapid and reliable AST results, significantly reducing time-to-result compared to conventional methods. Implementing this technology in routine microbiology practice could enable earlier optimization of antimicrobial therapy, thereby improving the management of Gram-negative BSIs and potentially reducing overall health care costs.

325 - Molecular surveillance of carbapenemase genes in critical care units: six years of Real-Time PCR experience

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Molecular surveillance of carbapenemase genes in critical care units: six years of Real-Time PCR experience

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Introduction The overuse of antibiotics has significantly contributed to the spread of antimicrobial resistance (AMR), allowing multi-drug-resistant bacteria, increasingly difficult to treat, to emerge. Guided by antimicrobial stewardship principles, this study aimed to monitor the presence of carbapenemase-producing bacteria in patients hospitalized in critical care settings.

Materials and Methods Between January 2019 and December 2024, a total of 18,633 rectal swabs were analyzed using the BD MAX Check-Points CPO Assay (BD MAX System), a qualitative Real-Time polymerase chain reaction (PCR) test targeting the blaKPC, blaNDM, blaVIM and blaOXA-48 genes. Samples were collected from patients admitted to the Hematology Ward, Intensive Care Unit (ICU), Emergency Medicine Ward, and Neonatal ICU of Azienda Ospedaliera di Rilievo Nazionale "San Giuseppe Moscati", Avellino, Campania Region.

Results Positivity rates were 2.30% in 2019, 5.26% in 2020, 2.03% in 2021, 2.64% in 2022, 3.35% in 2023 and 1.72% in 2024. KPC was the most frequently detected gene in all years, mainly in Hematology Ward and ICU. NDM, initially rare in 2020, showed a steady increase and was more prevalent in Emergency Medicine Ward and ICU in 2024. OXA-48, undetected in 2019, gradually increased and accounted for 9.5% of positive samples in 2024, especially in Emergency Medicine Ward. VIM peaked between 2021 and 2022 in ICU, and was also detected in Neonatal ICU in 2022 and 2024. In 2024, out of 63 positive cases, 40 were KPC (63.5%), 10 NDM (15.9%), 6 OXA-48 (9.5%), 5 VIM (7.9%) and 3 showed KPC/NDM co-resistance (4.8%).

Discussion and Conclusions Six years of molecular surveillance highlight the persistent circulation of KPC and the growing emergence of NDM and OXA-48 in high-risk settings. Real-Time PCR proved to be an effective tool for early detection and rapid containment. The increasing complexity of resistance patterns underlines the importance of strengthening infection control measures and applying targeted diagnostic and antimicrobial stewardship strategies. Including Neonatal ICU in the monitoring process enables timely identification of resistant strains even in vulnerable pediatric populations.

326 - Microbiological surveillance and antibiotic resistance models through syndromic panels in emergency settings: three-year analysis

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Microbiological surveillance and antibiotic resistance models through syndromic panels in emergency settings: three-year analysis

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INTRODUCTION

Syndromic panels represent a diagnostic revolution for the rapid identification of pathogens and determinants of antibiotic resistance, offering crucial implications for microbiological surveillance and public health. This study focuses on the epidemiological analysis of etiological agents and antibiotic resistance mechanisms detected in a high-flow hospital context, with the aim of evaluating their impact on infection control strategies and therapeutic optimization.

MATERIALS AND METHODS

2,187 samples processed with BioFire® FilmArray® systems (2022–2024) at the Polyclinic of Catania were retrospectively analyzed. The panels used included targets for respiratory, gastrointestinal, central nervous system and blood culture pathogens. Antimicrobial resistance genes are present in some syndromic tests. The microbiological data were stratified by year, sample type and department of origin, with statistical analysis of temporal trends and the distribution of resistance determinants. Particular attention was paid to samples from the emergency medicine department.

RESULTS

Over the three-year period, 1,218 pathogens were identified, with prevalences for Klebsiella pneumoniae, Escherichia coli, and Staphylococcus aureus. The most frequent resistance genes included blaCTX-M and mecA. A 15% annual increase in the detection of MDR pathogens was observed, particularly in Emergency Room (ER) patients. The early use of resistance data allowed the timely start of targeted therapies in 78% of cases, with a 40% reduction in the empirical use of carbapenems compared to the pre-panel period. The analysis of 2,187 samples processed highlighted an exponential increase in use, with dominance of respiratory panels. The pneumonia and gastrointestinal panels showed upward trends, while the use of the blood culture panel plummeted due to the availability of other rapid alternatives. The agreement with the gold standard methods was variable, correlated with the nature of sample and the type of analysis. In gastrointestinal samples, parasitological analysis underlines the importance of traditional microscopy. In the 366 samples from the emergency room, positivity to BioFire® highlighted a 37% reduction in the average time, direct discharges from the ER, less use of high intensity departments despite higher average National Early Warning Scores.

DISCUSSION AND CONCLUSIONS

The study confirms the value of syndromic panels as active surveillance tools for the emergence of resistance, while highlighting critical issues in concordance with traditional methods for pneumonia and blood cultures. The targeted use in the winter months for respiratory infections and the integration with clinical parameters optimize the microbiological impact factor, reducing therapeutic empiricism. We propose the implementation of diagnostic algorithms differentiated by syndrome, with mandatory culture validation for low concordance panels, maximizing adherence to antimicrobial stewardship programs.

327 - Fourier Transform Infrared Spectroscopy as a new approach to investigate the evolution of Pseudomonas aeruginosa strains colonisation/infection in Intensive Care Unit patients

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Fourier Transform Infrared Spectroscopy as a new approach to investigate the evolution of *Pseudomonas aeruginosa* strains colonisation/infection in Intensive Care Unit patients

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Background

Pseudomonas aeruginosa is an opportunistic pathogen and a primary cause of hospital infections, particularly in intensive care units (ICUs). This study aims to examine clonal relationships among *P. aeruginosa* strains from different patients, including strains from the same patient isolated at different times, and investigate phenotypic changes during the infection.

Methods

63 strains of *P. aeruginosa* isolated from ICU patients were subjected to PCR assays to assess virulence factors. Antimicrobial susceptibilities were performed with a broth microdilution test. Clonal relationship among *P. aeruginosa* isolates was evaluated using Fourier Transform-Infrared (FT-IR) spectroscopy technology.

Results

PCR resulted in a predominance of *exoT* gene (66%), followed by *toxA* (57%), *exoU* (44%), *exoY* (41%), and *exoS* (39%). A multidrug-resistant phenotype was detected among 18% of isolates. Strains displayed resistance in this order: meropenem (68%); ciprofloxacin (20%); levofloxacin (19%); ceftazidime (17%); amikacin (0%). Hierarchical clustering included a total of 48 isolates coming from 16 different patients, each of which was subjected to serial sampling with an average interval of approximately 7 days. The results obtained with an unsupervised analysis revealed, in all cases, that intra-patient isolates are grouped within the same phylogenetic cluster, showing a high genomic homogeneity. In 14 patients (87,5%), strains resulted phylogenetically identical with minimal or no branching between isolates, consistent with the persistence of a single clone over time. In two patients, although greater variability is present, isolates were still distributed within the same phylogenetic branch, suggesting an intra-host process of evolution rather than re-infection events. No patients showed isolates distributed in distant clusters, thus excluding heterologous reinfection.

Conclusion

P. aeruginosa strains appeared prone to variation in virulence factors during the evolution of the infection. Only 15 isolates, from 8 patients, maintained a stable profile. Strains coming from the same patient demonstrated a propensity to beta-lactam susceptibility variations. Simultaneously, fluoroquinolones and aminoglycosides susceptibility profiles remained unaffected. FT-IR spectroscopy succeeded in investigating clonal relationships. Overall, the data indicated a marked genetic stability of the strains over time, consistent with a persistent colonisation pattern. Isolates from different patients occasionally clustered, suggesting dissemination of prevalent clonal lineages or potential cross-transmission. These results underscore the usefulness of FT-IR spectroscopy as a high-throughput tool for epidemiological and clinical typing.

334 - Epidemiological patterns of respiratory pathogens across age, gender, and season during the post-COVID era: a two-year study

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Epidemiological patterns of respiratory pathogens across age, gender, and season during the post-COVID era: a two-year study

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Introduction

The aim of the study was to provide a comprehensive description of the incidence of respiratory infection in hospitalized patients with suspicion of acute respiratory infections (ARIs) across age, gender, and season during the post-pandemic era.

Materials and Methods

Nasopharyngeal specimens were collected and QIAstat-Dx® Respiratory SARS-CoV-2 Panel was used.

Results

During a 23-month period (June 2023-April 2025) 1750 respiratory specimens collected from hospitalized patients with suspicion of ARIs were retrospectively analyzed. The sample exhibited a male-to-female ratio of 54.5%, with most subjects being children (71.6%) and a smaller proportion being adults (28.4%).

In total, 1075 (65.3%) samples were found to be positive for at least one pathogen. The most prevalent pathogens detected were Rhinovirus/Enterovirus (HRV/EV) (60.1%), Respiratory syncytial virus (RSV,18.7%), Flu virus A/B (A,H1N1/2009,H1,H3,B) (10.2%), Adenovirus (Adv,9.2%), Human Metapneumovirus A/B (hMPV,8.5%). A higher percentage of positive results was observed in the pediatric compared to the adult group (84.7%and15.3%, respectively; $p<0.0001$).

Among the positive samples, 293 (27.3%) exhibited coinfections. Specifically, 82.3% of the cases exhibited interviral, 17.4% viral-bacterial, and 0.3% interbacterial infection. The most

frequent associations were observed between HRV/EV and Adv (16.7%) as well as between HRV/EV and RSV (16.3%). No differences were observed in the frequency of interviral and viral-bacterial infections between pediatric and adult groups. The sole inter-bacterial infection documented was observed within the pediatric group. A higher percentage of coinfections was observed in the pediatric compared to the adult group (29.8% and 13.4%,respectively; $p<0.0001$) as well as in males than in females (58.4% and 41.6%,respectively; $p=0.0465$).
Finally, seasonal analysis showed clear peaks: HRV/EV in autumn and spring, Flu viruses A/B in winter, RSV in autumn and winter, hMPV in winter and spring. Interestingly, a higher percentage of Flu virus A/B positive specimens were observed in winter 2024/25 compared those observed in winter 2023/24 (9.5%and24.5%, respectively; $p<0.0001$). In contrast to Flu A/H1N1/2009, which was observed in both winters, a peak of Flu A/H3 was exclusively detected during the winter of 2024/25.

Discussion and Conclusions

The use of syndromic panel testing has showed a high number of coinfections, especially in males and in the pediatric population. The findings provide crucial insights into the prevalence and seasonal distribution of respiratory pathogens, offering valuable data to inform public health strategies in the post-pandemic era.

336 - Prevalence of HPV and Other STIs in Men Who Have Sex with Men Implications for Screening Programs

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Prevalence of HPV and Other STIs in Men Who Have Sex with Men: Implications for Screening Programs

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INTRODUCTION. The incidence of Sexually Transmitted Infections (STIs) has increased worldwide since 2020. Men who have sex with men (MSM) are at a higher risk for HIV, HPV, and other STIs compared to the heterosexual population. Human papillomavirus (HPV), often overlooked in men, can lead to malignant lesions in the anogenital and head-and-neck regions if persistent. HIV-positive, as a high-risk group, exhibit a higher incidence of HPV-related anal cancer. For these reasons, we analysed the prevalence of STIs (HPV, *Treponema pallidum*, *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Ureaplasma parvum)* in a cohort of HIV-positive and HIV-negative MSM.

MATERIALS AND METHODS. T**hirty-nine MSM** presenting with a history of risky sexual behaviour at the Infectious Diseases Unit of Polyclinic Hospital "P. Giaccone" of Palermo were enrolled. **Rectal swabs and blood samples** were collected and subsequently sent to the Microbiology and Virology Unit at the same hospital. **Blood samples were tested for syphilis status**, while rectal swabs underwent two PCR assays: **HPV28 Detection Assay** for HPV detection and **STI Essential Assay Allplex™ (Seegene, Korea)** to screen other specified pathogens.

RESULTS. The most prevalent pathogen was HPV, detected in 82% of cases (32/39), followed by syphilis with a rate of 71.4% (30/39). Among HIV-positives, HPV was found in 85.7% of rectal swabs, while a slightly lower rate (77.8%) in HIV-negatives. Regarding syphilis, 71.4% of patients were positive in the HIV-positive (40% with RPR≥4) and 83.3% among HIV-negative (73.3% with RPR≥4). Considering the entire cohort, 41% (16/39) were positive for at least one

of the seven pathogens of the syndromic test: *M. hominis* and *U. urealyticum* were the most frequent (50%). In the HIV-positive, 47.6% were positive for at least one of the pathogens.

DISCUSSION AND CONCLUSIONS. The high prevalence of HPV in our cohort is particularly concerning, given its established link to anogenital and head and neck cancers, especially in at-risk populations such as HIV-positive individuals. Although our results showed no significant differences in the prevalence of HPV or syphilis between HIV-positive and HIV-negative populations, the high rates of co-infections, which increase the risk of developing HPV-related cancers, highlight the need for screening programs for at-risk groups such as MSM. This would allow for early identification of silently infected individuals and thus timely intervention and patient management. Screening would not only facilitate the detection of HPV-related lesions, such as anal dysplasia, but would also identify other sexually transmitted diseases that can significantly impair a patient's health and quality of life.

342 - Escherichia coli ST131: Application of Fourier Transform Infrared (FTIR) Spectroscopy for Rapid Detection and Typing in Healthcare Settings

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Escherichia coli ST131: Application of Fourier Transform Infrared (FTIR) Spectroscopy for Rapid Detection and Typing in Healthcare Settings

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Introduction
Escherichia coli ST131, a virulent and multidrug-resistant clone from phylogroup B2, is associated with severe infections and often carries the $bla_{\text{CTX-M-15}}$ gene, conferring resistance to critical antibiotics. Its global spread demands continuous surveillance. This study evaluates Fourier Transform Infrared (FT-IR) spectroscopy as a rapid, cost-effective and accurate method for identifying and differentiating ST131 strains, supporting its potential in clinical diagnostics.

Materials and Methods
Fifty-four *E. coli* strains from different wards of the University Hospital of Verona were analysed by IR-Byotyper (Bruker Daltonics, Germany), each tested in quadruplicate. Spectra were processed with infrared transmission spectrometers and OPUS software. Phylo-typing was performed using the Clermont method. DNA libraries of 14 strains were prepared with the Illumina DNA Prep Kit, and samples were analysed by Next-Generation Sequencing on the MiSeq instrument in paired-end mode (2 x 300 bp). Sequencing data were analysed with DRAGEN FastQC and with MultiQC v3.6.3. Reads assembly was performed with Velvet v1.2.10.

Results
Phylogenetic analysis of 54 isolates assigned 34 to phylogroup B2, 2 to A, 8 to A/ C, 8 to D/ E, and 2 to F. The core genome Multilocus Sequence typing (cgMLST) of the 14 strains that we performed, also whole genome, showed different ST, namely ST131 (8 strains), ST83, ST354, ST538, ST10, ST925, and ST69. All ST131 belong to the phylogroup B2, together with ST538; all other ST belong to different phylogroups. FT-IR spectroscopy identified different clusters. The IR Biotyper with an unsupervised method groups the strains belonging to the same phylogroup, like the two strains belonging to F, or strains belonging to D/E and A/C. The 34 strains belonging

to the phylogroup B2 are distributed in 4 different clusters, containing only the B2 phylogroup and ST131 strains.

Discussion and Conclusions

Phylogroup B2, especially ST131 strains, predominated among isolates and clusters, indicating ongoing intra-hospital transmission across wards and times. FTIR spectroscopy efficiently grouped phenotypically similar strains, proving a rapid and cost-effective complement to genomic typing for outbreak detection. These results support combining FTIR with phylo-typing to enhance surveillance and control of high-risk *E. coli* strains.

09 9. One Health

2 - Dati preliminari sulla sieroprevalenza dell'infezione da Rift Valley virus e Crimea Congo virus nelle popolazioni migranti in Italia

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Dati preliminari sulla sieroprevalenza dell'infezione da Rift Valley virus e Crimean Congo virus nelle popolazioni migranti in Italia

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Introduzione:

Il virus della febbre emorragica di Crimea-Congo (CCHFV) è l'agente eziologico della febbre emorragica di Crimea-Congo. La CCHF è una zoonosi caratterizzata da un quadro clinico variabile che va da una sintomatologia simil-influenzale non grave a emorragie interne e può culminare in insufficienza multiorgano, shock e morte.
Recentemente, uno studio di sieroprevalenza sui bovini in Italia ha rilevato una positività IgG al CCHFV dell'1,89% (15/794).
L'infezione della Rift Valley è una malattia che rientra tra gli otto patogeni emergenti con il potenziale di epidemie con gravi conseguenze economiche e sanitarie.
Il virus della Rift Valley (RVFV) è responsabile di una malattia virale zoonotica trasmessa dalle zanzare che colpisce animali ed esseri umani.
Diversi studi sono stati condotti in Africa e in Arabia Saudita con una sieroprevalenza dell'1,8% per gli anticorpi IgG in Kenya, del 2,1% in Tanzania e dell'11,1% in Arabia Saudita. I risultati di studi di sieroprevalenza nell'uomo condotti in Paesi limitrofi al bacino del Mediterraneo, che non hanno mai riportato casi di malattia, descrivono inoltre la presenza di sieroprevalenza nell'uomo di anticorpi IgG pari all'1,37% e in Tunisia e solo IgG pari al 4,9% in Turchia.

Metodi:
È stato utilizzato un questionario per valutare i potenziali fattori di rischio di infezione da RVF e CCHF. I sieri sono stati raccolti da 71 popolazioni migranti in Italia provenienti da regioni endemiche dell'Africa per la Rift Valley e la CCHFV sono stati eseguiti utilizzando test diagnostici di infezione indiretta IFA (indirect Immunofluorescent assay) su soggetti asintomatici con un fattore di rischio per l'esposizione all'infezione da CCHFV e RVFV.

Per i soggetti IgM positivi, la presenza del virus nel siero sarà valutata mediante PCR in tempo reale.

Risultati e conclusioni:
La sieroprevalenza nei campioni di migranti è stata significativamente più alta per il CCHFV (8/71, 11,2%) rispetto al RVFV (4/71, 5,6%). Tutte le IgM per entrambi i virus sono risultate negative. Il titolo dell'IFA è risultato compreso tra 1:20 e 1:40 per il CCHFV e tra 1:20 e 1,80 per il RVFV.
I volontari africani risultati positivi per le IgG RVFV provengono da Mali e Senegal; invece i positivi per le IgG CCHFV provengono da Gambia, Senegal, Nigeria, Egitto e Somalia.
Inoltre, un campione proveniente dal Senegal è risultato positivo alle IgG CCHFV e RVFV.
Questi dati preliminari hanno evidenziato l'importanza di attivare un sistema di sorveglianza nella popolazione africana per monitorare e mitigare il rischio di epidemie da RVF e CCHFV nei Paesi africani e in Italia.

3 - SARS-CoV-2 whole genome detection in faecal samples of free-ranging and alive Mediterranean sperm whales (Physeter macrocephalus) into the One Health concept

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SARS-CoV-2 whole genome detection in faecal samples of free-ranging and alive Mediterranean sperm whales (*Physeter macrocephalus*) into the One Health concept

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Introduction: Since SARS-CoV-2 has become endemic in humans, one of the greatest threats to public health was the resurgence of more virulent and transmissible variants. The considerable pathogen pressure imposed by the pandemic has caused concern about whether SARS-CoV-2 spilled into wildlife populations, established a sylvatic cycle, and potentially served as a source for new variants. To date, data on SARS-CoV-2 epidemiology and its circulating variants from the terrestrial environment are available, while those from the marine environment remain still scare. This study aimed to investigate SARS-CoV-2 presence and its variants in wild marine mammals within a One Health perspective. Moreover, the association with zoonotic gastrointestinal pathogens, previously detected in sperm whale faecal samples, were analysed and discussed.

Material and methods: As part of a long-term research project on marine mammal ecology, fecal samples from seventeen free ranging and alive Mediterranean sperm whales (*Physeter macrocephalus*) were collected in the Pelagos Sanctuary (North-Western Mediterranean Sea) between 2019 and 2023, by the Tethys Research Institute. Samples were subjected to Allplex™ SARS-CoV-2 Assay Real Time PCR and then to CleanPlex™ SARS-CoV-2 panel whole genome sequencing by Miseq Illumina platform.

Results: Out of 17 faecal samples analysed, 2 (11.8%) tested positive for the SARS-CoV-2 Assay Real Time PCR and two omicron variants were identified after whole genome sequencing. The two samples were co-infected with zoonotic *Giardia duodenalis* Assemblage A and *Cryptosporidium parvum* IIa subtype family, previously detected in the sperm whale faecal samples.

Discussion: This study represents the first detection of SARS-CoV-2 whole genome in faecal samples from free-ranging, live sperm whales adding data on the SARS-CoV-2 epidemiology from marine wildlife and highlighted the need for further investigation of coronaviruses in marine ecosystem. Moreover, these results give an overview of zoonotic microorganisms circulating in Mediterranean marine megafauna and shared with human, indicating more integrated multidisciplinary researches within the One Health approach to better understand

the associated risk and to prevent human and marine ecosystem exposure to these zoonotic and anthropozonotic microorganisms.

9 - Preliminary assessment of wild boar as carriers of multidrug-resistant E. coli strains.

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Preliminary assessment of wild boar as carriers of multidrug-resistant *E. coli* strains

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Introduction The wild boar (*Sus scrofa*), a widely distributed mammal found across diverse habitats, is gaining recognition for its role as a bioindicator in monitoring zoonotic and non-zoonotic diseases. Its significance is particularly evident in the context of antimicrobial resistance (AMR) dynamics, where it serves as a valuable model for studying the emergence, spread, and persistence of AMR at the interface of human, livestock, and wildlife populations. This study investigated the potential of wild boar (*Sus scrofa*) as sentinel species to monitor AMR, by evaluating the phenotypic and genotypic antimicrobial resistance profiles of *E. coli* strains isolated from nasal swabs of healthy wild boars. **Materials and Methods** The nasal swabs of wild boars were collected at the time of capture, during the hunting season 2022 in Campania Region (Southern Italy). *E. coli* isolation was carried out by using Mac Conkey agar (MCA) plates, a selective and differential medium for Gram-negative bacteria. Once the bacteria revealed themselves on MCA plates, suspected lactose fermenting *E. coli* colonies were identified by using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI–TOF MS). The antimicrobial resistance profiles of the isolated strains were evaluated against 12 antimicrobials belonging to 8 different classes by disk diffusion method on Mueller Hinton agar plates. PCR assay was performed to detect extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) genes. **Results** From 150 nasal swabs, 112 Gram-negative bacteria were isolated, with 78.6% (88/112) identified as *E. coli*. Notably, antimicrobial resistance profiling revealed high resistance rates to erythromycin (93.2%) and ampicillin (83.0%), and resistance levels higher than 50.0% to amoxicillin-clavulanate and cefalexin (both 58.0%), tetracycline (55.7%), and imipenem (53.4%). Regarding ESBL genotypic resistance, bla_{PER} was the most prevalent gene (13.6%; 12/88),

followed by *bla*$_{SHV}$ (11.3%; 10/88), *bla*$_{TEM}$ (4.5%; 4/88), and *bla*$_{CTX-M}$ (3.4%; 3/88). MBL-genotypic resistance was due mainly to *bla*$_{IMP}$ (38.6%; 34/88), followed by *bla*$_{VIM}$ (28.4%; 25/88), *bla*$_{NDM}$ (17.0%; 15/88), with *bla*$_{GES}$ and *bla*$_{OXA-48}$ both detected in 14.8% of tested strains (13/88). **Discussion and Conclusions** The observed elevated levels of resistance to antibiotics commonly used in veterinary medicine are concerning, especially considering that wild boars are not typically subject to antimicrobial selective pressure. Therefore, this research highlights the necessity for continuous monitoring of wild fauna, such as wild boars, which can act as reservoirs for multidrug-resistant bacteria and potentially contribute to their dissemination across humans, animals, and the environment.

10 - Citrobacter spp.-associated and non-associated lesion in a stranded loggerhead sea turtle (Caretta caretta) from Italy.

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Citrobacter spp.-associated and non-associated lesion in a stranded loggerhead sea turtle (_Caretta caretta_) from Italy.

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Introduction The skin of turtles, particularly aquatic species, can harbor a diverse range of bacteria, including *Citrobacter* species. Consequently, these animals may act as reservoirs of pathogenic and multidrug-resistant bacteria, posing a potential public health concern. This study investigated the presence and role of *Citrobacter* spp. in lesions found on the carapace, plastron, and skin, and also in healthy anatomical districts of a loggerhead sea turtle (*Caretta caretta*) hosted at the Livorno Aquarium of the Toscana Region, Italy. **Material and Methods** Sampling was performed by swabbing the lesions, oral cavity, and cloaca. In total, nine swabs were collected: four from the plastron lesions, one from a central lesion on the carapace, one from an ulcerated nuchal mass, one from the oral cavity and two cloacal swabs. Once delivered in the laboratory of the Department of Veterinary Sciences of the University of Pisa, specimens were seeded on selective solid media for *Enterobacteriaceae*. Colonies from pure cultures were subjected to rapid screening tests and stored at -20°C for further studies. The identification of the isolates and the evaluation of their antimicrobial resistance profiles was carried out at the Bacteriology Laboratory of the Department of Veterinary Medicine and Animal Production, University of Naples "Federico II". Bacterial identification was by using the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI TOF MS). Then, the identified strains were tested for their susceptibility to 12 antimicrobials, belonging to nine antimicrobial classes by disc diffusion method on Mueller Hinton agar plates. **Results** 15 strains were recovered from the samples analyzed and resulted to belong to *Critrobacter* spp. In particular, *Citrobacter braakii* was the most frequently isolated species (73.3%) from all the collected samples, followed by *Citrobacter freundii* (26.7%) recovered only from oral and cloacal samples. The evaluation of antimicrobial resistance profiles revealed a total resistance to ampicillin (100%), followed by imipenem (93.3%), tetracycline (53.3%), cefalexin and piperacillin-tazobactam both with a value of 46.7%, and meropenem (40%). Worryingly, 13/15 strains (86.7%) were multidrug-resistant. **Discussion and Conclusions** The findings of this study demonstrate the presence of *Citrobacter* spp., an opportunistic pathogen, with a notable

prevalence of multidrug-resistant strains, in a loggerhead sea turtle in Italy, across both lesioned and healthy anatomical locations. Further research is crucial to fully elucidate the pathogenic role of these bacteria in sea turtles and to assess the broader ecological and public health implications of antimicrobial resistance in this vulnerable animal.

11 - Investigating Staphylococcus spp sharing between dogs and owners in two Italian Veterinary Hospitals: a preliminary study.

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Investigating *Staphylococcus* spp sharing between dogs and owners in two Italian Veterinary Hospitals: a preliminary study.

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Introduction *Staphylococcus spp.*, common commensals of the skin and mucous membranes in humans and animals, are also significant pathogens due to their diverse virulence factors and antimicrobial resistance. This preliminary study investigated the presence of these bacteria in dogs and their owners by analyzing nasal swabs collected from anesthetized dogs during surgery to minimize stress. The aim of this study was to identify and characterize nasal *Staphylococcus* spp. to assess the correlation between human and canine compositions, comparing owners and their dogs at two Veterinary Teaching Hospitals in Northern (Turin) and Southern Italy (Naples). **Materials and Methods** Nasal swabs, collected from dogs and their owners in each Veterinary Teaching Hospital, were streaked on Mannitol Salt Agar plates, a selective and differential medium for *Staphylococcus* spp. After an overnight incubation, the recovered colonies were first evaluated for their morphologic and fermentative features and subjected to rapid standard screening techniques. Subsequently, bacterial identification was carried out by using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI–TOF MS). **Results** Approximately 100 dog-owner pairs were sampled at each hospital. A low concordance (8%) of the same *Staphylococcus* species between owners and dogs was observed in Naples, significantly lower ($p < 0.05$) than the higher concordance (27%) found in Turin. The most frequent isolates from dogs in Naples were *Staphylococcus pseudintermedius* (n=24) and *Staphylococcus aureus* (n=11), while in owners, *Staphylococcus epidermidis* (n=52) and *Staphylococcus aureus* (n=24) were the most common. The same *Staphylococcus* species was found in eight dog-owner pairs: *S. aureus* (four pairs), *S.*

epidermidis (three), and *S. warneri* (one). In Turin, the most frequent *Staphylococcus species* from dogs were *S. pseudintermedius* (n=19) and *S. aureus* (n=17), followed by *S. intermedius* and *S. capitis* (n=9). In owners, *S. aureus* (n=36) and *S. epidermidis* (n=32) were the most prevalent. The same *Staphylococcus* species was identified in 24 dog-owner pairs: *S. aureus* (eleven pairs), *S. epidermidis* (four), *S. pseudintermedius* (three), and less frequently *S. capitis* (two), *S. haemolyticus*, *S. intermedius*, *S. warneri*, and *S. xylosus* (one pair each). **Discussion and Conclusions** In conclusion, this study highlights the importance of *S. aureus* and *S. epidermidis*, primary human microbiota colonizers, in dog-owner pairs, suggesting a higher likelihood of human-to-animal transmission. However, the higher correlation observed in Turin compared to Naples warrants further investigation into these regional differences.

14 - Hantaviruses infection: preliminary data of viruses seroprevalence in Italy

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Hantaviruses infection: preliminary data of viruses seroprevalence in Italy.

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Introduction:

Hantaviruses are negative-sense, single-stranded RNA viruses that belong to the order *Bunyavirales* and family *Hantaviridae*. The family *Hantaviridae* includes 7 genera and at least 28 species with a high distribution worldwide. The wide variability is due to the close co-evolution between the virus and its primary host, mainly represented by rodents, that are reservoirs of various mammalian hantaviruses. Humans could be infected with mammalian hantaviruses by inhaling viral particles derived from rodents' excrement, by eating contaminated food or by direct contact with rodents' urine or excrements. Hantiviruses' human infection can cause pulmonary or renal failure with a high mortality rate. The last European report conducted by ECDC confirmed the presence of hantaviruses in 28 different European country. A few data are known about hantaviruses prevalence in Italy. The climate change of

the last 10 years could cause a demographic explosion of the reservoir population in urban area causing more contact between reservoirs and humans. Despite is not present a specific Hantaviruses' surveillance plan on Italian territory, these viruses are tracked since 2018. The aim of this project is to enforce a surveillance system on Hantaviruses in all the Italian territory, considering the contiguity to territories where these viruses are endemic, mainly in at-risk categories.

Objectives:

Analyze the prevalence of Hantaviruses infection in all the Italian territory (North, Center and South) using two different indirect diagnostic tests.

Patients & Methods:
From December 2023 to March 2025 we serially collected serum sample from healthy donors belonging to at-risk categories (Veterinarians, farmers or people exposed to rodents). Serum samples were collected, cryopreserved and analyzed for the presence of anti-Hantaviruses IgM and IgG by Indirect Immunofluorescence assay (IFA) and Enzyme-Linked Immunosorbent Assay (ELISA). Positive IgM were analyzed by Real Time PCR. All the samples resulted positive in IgG and/or IgM in ELISA and/or IFA were confirmed using Immunoblot assay.

Results:
A total of 276 serum samples were analyzed.
Our preliminary data indicate that:
The serological prevalence of anti-Hantavirus IgG were 15,94% by IFA and 1,45% by ELISA.
The serological prevalence of anti-Hantavirus IgM were 4,76% by IFA and 4,83% by ELISA.
In total 5/276 were confirmed in IgG using Immunoblot assay (0.55%)
All samples positive IgM are resulted Real Time-PCR negative.

Conclusion:
Our findings confirmed the presence of Hantaviruses in our country, suggesting the importance of tracking and monitoring the seroprevalance of these viruses more closely.

26 - A Comprehensive Marker Database for Broad-Spectrum Metagenomic Exploration

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A Comprehensive Marker Database for Broad-Spectrum Metagenomic Exploration

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Introduction

Taxonomy classification of the bacteriome and virome of a metagenomic sample remains one of the most difficult challenges in metagenomic studies. This difficulty comes from a combination of factors, including the diverse heuristic approaches used by different classification tools and the limited genomic representation in the databases used to perform these analyses. Although MetaPhlAn has emerged as a reliable tool for taxonomic classification, its database is centred around the human microbiome. To address this limitation, a novel database compatible with MetaPhlAn has been constructed, incorporating most bacterial and viral genomes from the NCBI GenBank database.e

Materials and Methods

Genomic sequences of bacteria and viruses were retrieved from the GenBank FTP server and screened for completeness. Following genomes collection, skani was used to calculate the Average Nucleotide Identity (ANI) over all genomes. Genomes with ANI scores greater or equal to 99% have been deduplicated to eliminate redundant entries. The resulting ANI scores were then converted to a distance metric, and the genomes were clustered using UPGMA, an average linkage method. The clustering results have then been used to define species-, genus- and family-level genome bins (SGBs, GGBs and FGBs respectively) at the three selected thresholds of 95%, 85% and 70% identity, respectively. Finally, genomic regions were extracted and unique marker genes identified for each group. Up to five marker genes were selected per group; if it was not possible to identify at least one marker gene for a specific group, the group was merged with its nearest neighbour.

Results

This novel database extends MetaPhlAn's original functionality beyond the human microbiome. Its enhanced genomic coverage facilitates the detection of additional taxa absent in the standard MetaPhlAn database. Preliminary results show a comparable performance between the two databases. The novel database requires additional time to perform the analysis with respect to the default MetaPhlAn database, most likely due to the inclusion of additional markers. Nevertheless, the novel database is able to detect previously undetectable markers without loss of accuracy for most organisms.

Discussion

Although this new database enables the detection of a broader range of microbes, there is still a potential advantage of using a specialised database tailored for the specific sample type. Therefore, this database should serve as a robust starting point for more advanced analyses or to characterise samples with uncertain origins. Additionally, due to the impossibility of assigning unique markers to some clusters, it is possible that the novel database is able to detect the correct genus but not the specific species in certain scenarios.

45 - Innovative strategies against acute Pseudomonas aeruginosa infections: the potential of natural compounds

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Introduction: *Pseudomonas aeruginosa* is a resistant and highly persistent opportunistic pathogen, frequently implicated in healthcare-associated infections. Its pathogenicity is driven by a wide array of virulence factors, including biofilm formation, diverse types of motilities, and the production of toxins such as pigments and proteolytic enzymes. Given the rise in antibiotic resistance, there is an urgent need to explore alternative therapeutic strategies. Anti-virulence therapy represents a promising alternative, since by targeting the pathogen's virulence mechanisms rather than bacterial survival, this strategy is not expected to exert the same selective pressure as traditional antibiotics, thus reducing the risk of resistance development. In this context, the essential oils and their main components, have emerging as potential modulators of bacterial phenotypes, including virulence. In this research project, we investigated the antimicrobial and anti-virulence activities of carvacrol and thymol, two phenolic compounds found in *Coridothymus capitatus,* against clinical strains of *P. aeruginosa*, as well as their potential synergistic effect when combined with conventional antibiotics.
Materials and Methods: A collection of clinical *P. aeruginosa* strains was phenotypically characterized. First, the minimal inhibitory (MIC) and minimal bactericidal concentration (MBC) of carvacrol and thymol were determined. Sub-MIC concentrations of carvacrol and thymol were then used to assess the inhibition of biofilm formation, bacterial motility, pigment release, and extracellular protease production. The potential synergistic effect of carvacrol and thymol with conventional antibiotics was also evaluated using a checkerboard assay. A selection of chemical analogues of carvacrol and thymol were also analyzed.
Results: The obtained results showed that carvacrol possessed higher antimicrobial activity compared to thymol. Sub-MIC concentrations of both phenolic compounds effectively reduced biofilm formation, swimming and swarming motility, as well as the production of pyoverdine and phenazines (pyocyanin and pyorubin). A significant reduction in the release of extracellular proteases was also observed. Moreover, a synergistic effect was found between the molecules and ceftazidime/avibactam in multidrug-resistant (MDR) *P. aeruginosa* strains.
Discussion and Conclusions: The observed anti-virulence activity of carvacrol and thymol, along with their synergistic effects with conventional antibiotics, make them promising candidates as alternative treatments for acute infections caused by MDR *P. aeruginosa strains*. Further studies with analogues could be useful to elucidate the mechanism of action of phenolic compounds against virulence determinants of *P. aeruginosa*.

58 - Bacterial-derived extracellular vesicles: comparative evaluation of purification protocols

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Bacterial-derived extracellular vesicles: comparative evaluation of purification protocols

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1. Introduction

Bacterial Extracellular Vesicles (bEVs) are membranous nanostructures produced by both gram-negative and gram-positive bacteria. They are surrounded by a lipidic bilayer and contain different molecules (nucleic acids, proteins, etc.) that can be transferred to host cells and other bacteria, playing thus a relevant role in cell-to-cell communication. Importantly, bEVs are involved in host-pathogen interactions, by delivering resistance and virulence factors, possibly stimulating host immune responses. This is critical especially for infections induced by

antibiotic-resistant bacteria. In this context, resistant *Enterococcus* strains greatly contributes to mortality rates. The understanding of these novel mechanisms of interaction is thus essential for the development of new therapeutic approaches and the establishment of specific strategies for the infection management.

2. Materials and Methods

bEVs were isolated from *Enterococcus faecalis vancomycin-sensitive* (VSE, ATCC 29212 strain) using two protocols: i) ultracentrifugation (UC), and ii) size exclusion chromatography (SEC). The strain was inoculated in DMEM, grown overnight at 37 °C and harvested upon reaching the early exponential phase. Upon centrifugation at 8000 x g for 10 min, the supernatants were filtered (0.22 µm).

3. Results

After UC or SEC bEV isolation, the vesicles were analysed for size and concentration via Nanoparticle Tracking Analysis (NTA). As a control, the same workflow was applied to the medium alone. The analysis of size revealed that SEC-isolated bEVs are smaller compared to their respective controls, while UC-isolated bEVs are bigger. Interestingly, the number of bEVs isolated via SEC was significantly higher compared to both controls and UC-isolated EVs.

4. Discussion and Conclusions

The differences in terms of size and number of recovered vesicles suggest that SEC approach improves yield and quality of bEVs. Moreover, SEC-isolated EVs are less subjected to mechanical stress, allowing their use for downstream applications. Further analysis will be carried out to characterize the DNA and protein content of bEVs. Moreover, functional experiments will potentially elucidate their role in antimicrobial resistance and host-pathogen interactions.

88 - A sero-epidemiological analysis of Coxiella burnetii in AUSL Romagna from 2020 to 2024

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A sero-epidemiological analysis of Coxiella burnetii in AUSL Romagna from 2020 to 2024

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Introduction

Coxiella burnetii is a Gram-negative, obligate intracellular bacterium and the etiological agent of Q fever. The disease primarily affects individuals living or working in rural areas through the inhalation of aerosols contaminated with *C. burnetii*, often originating from placental materials, vaginal secretions, urine, or feces of infected animals. The main reservoirs include

domestic animals (such as cattle, sheep, and goats), wild animals, and arthropod vectors, particularly ticks.

This study aimed to assess the serological status of individuals potentially exposed to *C. burnetii* in the AUSL Romagna area between 2020 and 2024, using specific screening tests.

Materials and Methods

A total of 806 serum samples were analyzed using enzyme-linked immunosorbent assay (ELISA) to detect IgG and IgM anti-C. burnetii antibodies. Positive samples were subsequently confirmed by indirect immunofluorescence assay (IFA) to differentiate between antibodies IgG phase I and phase II antibodies and the titration, allowing for characterization of the infection stage.

Results

Results were classified as negative, equivocal, or positive. The majority of samples (95.3%) tested negative for both IgG and IgM. A total of 3.5% showed IgM positivity or equivocal results with negative IgG, while only 0.4% presented significant differences between phase I and phase II IgG levels. Individuals in the latter group were contacted for follow-up, which did not reveal any evidence of seroconversion or a clear predominance of phase II IgG (+++).

Discussion and Conclusions

C. burnetii is recognised as an important zoonotic pathogen that can be transmitted by several routes, including the respiratory and gastrointestinal tracts and direct contact with the skin and mucous membranes. The potential consequences include the development of endocarditis, vascular infection, bone and joint infection, lymphadenitis and interstitial lung disease. In addition to the conventional presentation, atypical infection may occur with involvement of the neurological system, eyes and other associated manifestations.The findings indicate a low incidence of active antibody response among the examined population, with an almost complete absence of significant seroconversion. These results contribute to the epidemiological assessment of the infectious risk associated with *C. burnetii* exposure in the AUSL Romagna area.

93 - Profiling the resistome and virulome of Bacillus strains used for probiotic-based sanitation: a multicenter WGS analysis

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Topic: One Health

Profiling the resistome and virulome of *Bacillus* strains used for probiotic-based sanitation: a multicenter WGS analysis

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Introduction: Healthcare-associated infections (HAIs) caused by microbes exhibiting antimicrobial resistance (AMR) represent an increasing and global threat to human health. Chemical disinfectants are used to reduce the presence of pathogens in the hospital environment, but they can favor the selection of resistant strains, potentially worsening the AMR concern. In the search for effective systems able to control bioburden without affecting this aspect, probiotic-based sanitation (PBS) including *Bacillus* probiotics was reported to provide stable reduction of pathogens, AMR, and associated HAIs. However, though *Bacillus* probiotics are classified as nonpathogenic, comprehensive data about the potential genetic alterations of these probiotics following prolonged contact with surrounding pathogens are not yet available. Our study aimed to assess in depth the genetic content of PBS-*Bacillus* isolates to evaluate any eventual variations that occurred during their usage.
Materials and Methods: PBS-*Bacillus* isolates, both from the original PBS detergent and from 172 environmental samples collected from eight hospitals sanitized with PBS over a 30-month period, were analyzed by whole-genome sequencing (WGS), providing a detailed profiling of

their SNPs, resistome, virulome, and mobilome, in order to verify their genetic stability, thereby validating their safety for use in associated cleaning systems.
Results: The two species *B. subtilis* and *B. velezensis* were identified in both the original product and the hospital environment, and SNP analysis revealed the presence of two clusters in each species. No virulence/resistance genes or mobile conjugative plasmids were detected in either the original PBS-*Bacillus* strain or any of the analyzed environmental isolates, confirming their high genetic stability and their low/no tendency to be involved in horizontal gene transfer events, even after prolonged contact with surrounding pathogens.
Discussion and Conclusions: The data obtained by metagenomic analysis revealed the absence of genetic sequences associated with PBS-*Bacillus* and the lack of alterations in all the environmental isolates analyzed, despite their continuous contact with surrounding pathogens. These results support the safety of the *Bacillus* species analyzed. Further metagenomic studies aimed at profiling the whole genomes of these and other species of *Bacillus*, possibly during longer periods and under stress conditions, would be of interest since they may provide further confirmation of their stability and safety.

95 - A Sustainable Combined Approach to Control the Microbial Bioburden in the School Environment

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Topic: One Health

A Sustainable Combined Approach to Control the Microbial Bioburden in the School Environment

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Introduction: The school environment represents the indoor space where students spend most of their time for many years, and an unhealthy indoor microbiome can significantly impact students' health. School rooms are often built with materials that may favor microbial contamination, can suffer from insufficient ventilation, and are mostly sanitized by chemical disinfectants, which are known to have temporary effects, to increase pollution, and to select drug-resistant microbial strains. By contrast, sanitation systems based on the use of eco-friendly detergents containing safe *Bacillus* probiotics (probiotic-based sanitation, PBS) have been reported to be effective in controlling the bioburden in sanitary and non-sanitary environments, while also preventing the increase in antimicrobial resistance (AMR) and pollution. Our study aimed to investigate the impact of an integrated sustainable approach, including the introduction of plants, ventilation, and PBS sanitation, on the indoor microbiome in an Italian high school, with the goal of creating a healthier environment for students and teachers.
Materials and Methods: A pre–post case–control study was performed in a high school in Ferrara (Italy), involving two classrooms with superimposable features, enrolled as test and control rooms (TR and CR). Over a year, three interventions were sequentially implemented and evaluated for their impact on surface and air bioburden: 1) introduction of plants, 2) introduction of mechanical ventilation system, 3) introduction of PBS in substitution of chemical disinfection. Microbial monitoring was performed bimonthly to assess microbes'

amount and AMR by both colony-forming unit (CFU) count and quantitative real time PCR microarrays. At each sampling campaign, surface (floor, teacher's desk, and two student tables) and air samples were collected both before and after students' classes. In parallel, environmental parameters (temperature, relative humidity, and CO_2) were monitored in continuum throughout the whole study.

Results: At baseline, a remarkable bioburden level was detected (around 12,000 and 20,000 CFU/m^2, before and after classes, respectively). *Staphylococcus* represented the prevalent bacterial genus. Fungi, mainly *Aspergillus* spp., were also detected. Several antibiotic-resistance genes (ARGs) were detected in the basal indoor microbiome, conferring resistance against beta-lactams, aminoglycosides, and macrolides. Compared to CR, a decrease in fungal contamination was observed in TR following the introduction of plants. However, the most significant decrease in pathogens and AMR was detected following the introduction of ventilation and PBS, which decreased pathogen level by >80% ($p < 0.001$) and AMR by up to 3 Log_{10} ($p < 0.001$) in TR compared to CR.

Discussion and Conclusions: Collected data support the use of combined strategies to improve indoor microbial quality and confirm that PBS can effectively contribute to controlling indoor bioburden and AMR spread in community high-traffic environments.

101 - Wastewater surveillance of Human Adenoviruses in Italy: quantification and molecular typing using digital PCR and Nanopore sequencing

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Wastewater surveillance of Human Adenoviruses in Italy: quantification and molecular typing using digital PCR and Nanopore sequencing

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Introduction Wastewater-based epidemiology (WBE) is increasingly recognized as a powerful tool to monitor viral pathogens circulating at the community level. Human adenoviruses (HAdVs) are widespread and environmentally persistent, yet data on genotype diversity in Italian wastewater are limited. This study aimed to quantify HAdVs in untreated wastewater and characterize their genetic diversity using digital PCR (dPCR) and sequencing.
Materials and Methods A total of 168 untreated wastewater samples were collected across Italy. Quantification of HAdVs was performed using dPCR. For molecular typing, nested PCR targeting a conserved region of the HAdV genome (Hexon) was employed, followed by Sanger sequencing for genotype identification. To improve resolution and detect rare types, long-read amplicon sequencing via Oxford Nanopore Technologies (ONT) was applied on pooled PCR products.
Results HAdV was detected in 93% of the analysed samples, with viral concentrations reaching up to 4.5×10^6 genome copies per litre. Overall, 118 samples (70.2%) were successfully amplified by nested PCR. Sanger sequencing produced high-quality sequences for 69 samples (58.5%), allowing the identification of four HAdV species: A, B, C, and F. Specifically, HAdV-B3 (45%) and HAdV-F41 (46%) were the most prevalent genotypes, followed by HAdV-A12 (3%) and HAdV-C1 (2%). Three additional samples showed identical matches to both HAdV-C2 and

HAdV-C89 prototypes, making precise typing impossible (4%). In 44 samples (37.3%), sequencing results were ambiguous due to mixed electropherogram signals, likely reflecting the presence of multiple co-circulating types. ONT sequencing confirmed Sanger results and revealed seven additional genotypes: HAdV-B21, C5, D45, D46, D49, D83, and F40.

Discussion and Conclusion These results highlight the widespread distribution of HAdV throughout Italy. Notably, the predominance of HAdV-B3 aligns with clinical respiratory data. In contrast, HAdV-F41, was widely detected in wastewater; however, it is not typically captured by respiratory clinical surveillance. This underscores the ability of WBE to provide a broader and more comprehensive picture of viral circulation, encompassing both respiratory and gastrointestinal infections, including asymptomatic cases. Despite pooled sequencing and single winter sampling limitations, our findings confirm the utility of WBE for monitoring viral circulation and genotype diversity. Future studies integrating individual sample sequencing and year-round sampling would further enhance our understanding of adenovirus epidemiology.

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103 - Integrated Genomic and phenotypic analysis of ST25 Acinetobacter baumannii Human and Animal isolates reveals clade-specific adaptations

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Integrated genomic and phenotypic analysis of ST25 *Acinetobacter baumannii* human and animal isolates reveals clade-specific adaptations

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Background

Acinetobacter baumannii is an opportunistic pathogen known for its high antimicrobial resistance and its ability to persist in hostile environmental conditions. Previous studies have demonstrated that genomes assigned to ST25 lineage shared high genomic similarity among isolates from humans and animals and clustered in four clades (I, II, III, IV), with clade IV further subdivided into IVa, IVb, IVc, and IVd. The aim of the present study is to perform an integrated genomic and phenotypic analysis of ST25 isolates from both human and animal origins.

Materials and Methods

We actualized the phylogenetic analysis of 203 ST25 *A. baumannii* genomes from both human (148) and animal (55) sources were retrieved from the NCBI database. A core genome MLST analysis (pyMLST) was performed. Capsule (KL) and lipooligosaccharide (OCL) loci were assigned using the Kaptive software. A representative subset of 41 isolates, belonging to each clade, was selected for phenotypic testing, including biofilm formation, virulence in the *Galleria mellonella* model, resistance to different concentrations (50-250μM) of hydrogen peroxide, desiccation survival for 60 days, and serum resistance. *A. baumannii* ACICU (ST2) and AYE (ST1) were tested as well.

Results

KL14 was the predominant capsular locus (47%), mostly found in clades II (20%) and IV (80%), followed by KL139 (10.3%). OCL6 was the most frequent OCL type (50%), followed by OCL5 (35%). Among the 41 ST25 isolates, only 19 were weak biofilm producers and there was no correlation between biofilm formation and assigned KL/OCL types. In virulence assays, the isolates of clades IVb and IVd showed the highest virulence (LD50 of 6.8 and 1.5 $\times 10^6$ CFU/mL, respectively) in *Galleria mellonella* model, while clades II, III, and IVc exhibited intermediate virulence compared to positive (ACICU) and negative (AYE) control, with LD50 values of 7.7, 1.2, and 1.5 $\times 10^7$ CFU/mL, respectively. Hydrogen peroxide resistance varied among clades. Clade III and IVc isolates showed reduced survival; in contrast, clades II, IVb, and IVd maintained high levels of survival (~10^6 CFU/mL) up to 6 hours. In desiccation survival tests, isolates of clades II and IV showed 100% viability (10^3 CFU/mL) at 30 days, with complete loss of viability by day 40. Clades III, IVb and IVd exhibited prolonged survival, with 100%, 85% and 54% viability at 30 days (10^3, 10^3 and 10^2 CFU/mL, respectively) and complete mortality by day 50. In addition, IVc isolates showed lower serum resistance, with a survival of 1×10^4 CFU/mL at 45 minutes, compared with clades II, III, IVb and IVd, which showed higher survival (~1×10^7 CFU/mL at 45 minutes).

Conclusions

Genomic and phenotypic analyses revealed significant heterogeneity among ST25 *A. baumannii* clades. In particular, clade IVb and IVd showed highest virulence and elevated resistance to serum and desiccation, clade II showed elevated resistance to serum and hydrogen peroxide, clade IVc combined intermediate virulence with low hydrogen peroxide and serum resistance, but high desiccation tolerance. Importantly, phenotypic differences reflected clade identity rather than isolate source, suggesting that phenotypic features contribute to the environmental persistence and pathogenic potential of *A. baumannii* ST25 isolates.

106 - Mapping clinically significant ARGs in Italian wastewater: distribution, persistence and seasonal dynamics

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Mapping clinically significant ARGs in Italian wastewater: distribution, persistence and seasonal dynamics

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Introduction Antibiotic resistance is a growing threat across human, animal and environmental sectors. Wastewater treatment plants (WWTPs) are reservoirs of antibiotic resistance genes (ARGs), which are not fully eliminated before water is discharged into receiving bodies. This study examines the distribution of clinically relevant ARGs across influent and effluent wastewater in Italy.
Materials and Methods A total of 38 wastewater samples (19 influents and 19 effluents) were collected during 2024 from WWTPs located in Piedmont (North), Lazio (Central), and Sicily (South). The selected ARGs confer resistance to multiple clinically relevant antibiotic classes, including beta-lactamases (*bla*$_{\text{OXA48}}$, *bla*$_{\text{CTXM1 group}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$), sulphonamides (*sul1*), tetracycline (*tetA*), vancomycin (*vanA*, *vanB*) and methicillin (*mecA*, *mecC*). The integrase gene *Int1*, a mobile genetic element, was also quantified. ARGs abundance was measured by digital PCR, both in absolute terms and normalised to 16S rRNA gene copies/L (g.c./L), to account for differences in bacterial load.
Results All samples tested positive for one or more ARGs, except for *mecC*, which was consistently undetected. The mean concentrations (g.c./L) in untreated wastewater, ranked by absolute abundance, were: *Int1* (5.0×10^9), *sul1* (3.4×10^9), *tetA* (7.6×10^8), *bla*$_{\text{TEM}}$ (5.0×10^8), *bla*$_{\text{NDM}}$ (2.6×10^8), *vanA* (2.6×10^8), *mecA* (1.9×10^8), *bla*$_{\text{CTX-M-1 group}}$ (1.0×10^7), *bla*$_{\text{OXA-48}}$, (7.3×10^6), *bla*$_{\text{KPC}}$ (3.2×10^6) and *vanB* (1.7×10^6). Mean concentrations in treated effluents showed reductions from one to two log units. After normalization to the 16S rRNA gene, 90 out 228 (~ 40%) measurements exhibited an increase in relative abundance of ARGs in effluents (e.g .

bla$_{OXA-48}$ and *bla*$_{TEM}$). Geographycally, *bla*$_{CTX-M-1\ group}$ and *tetA* were most prevalent in Southern Italy, while *bla*$_{NDM}$ was more abundant in $_{the}$ North. Other ARGs showed similar distribution pattern. Most ARGs showed minimal seasonal variation, with some exceptions: *tetA*, *Int1* and *bla*$_{CTX-M-1\ group}$ increased in spring while *bla*$_{NDM}$ and *vanA* increased in spring/summer.

Discussion and Conclusions Regional and sample-level differences in ARG distribution likely reflect multiple factors, including antibiotic consumption patterns and population density. Consistent with previous findings, ARGs persisted after treatment, raising concerns about their potential recirculation to humans through water systems or other pathways. Future efforts should focus on identifying the drivers of ARG persistence and developing strategies to mitigate their environmental dissemination.

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107 - Evaluation of a hybrid capture-based sequencing panel for comprehensive detection of antibiotic resistance genes in wastewater

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Evaluation of a hybrid capture-based sequencing panel for comprehensive detection of antibiotic resistance genes in wastewater

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Introduction Antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) in wastewater are of critical concern due to their potential to spread into natural ecosystems and pose risks to public health. This study evaluates a sequencing strategy based on a comprehensive multi-target enrichment probes panel for the detection of ARGs wastewater.
Materials and Methods Eight influent and effluent wastewater samples were collected quarterly over a year from a central Italian wastewater treatment plant. ARG enrichment was performed using the QIAseq xHYB AMR panel (Qiagen) and enriched targets were sequenced with Illumina short reads technology. The QMI-AR was used for ARGs identification.
Results The analysis of the 8 samples identified 743 (26.6%) out of 2,786 ARGs included in the panel, which are associated with 35 distinct antibiotic resistance classes. Out of 743 ARGs, 81 (11%) were exclusively detected in influents, 38 (5%) were found in effluents, while a total of 624 (84%) were detected in both influents and effluents. For each season, approximately 50% of the relative abundance (RA) was accounted for by a small group of dominant ARGs. In winter, the most abundant ARGs were *tet*(Q), conferring resistance to tetracycline, *ant(3'')-Ia* to aminoglycoside, bla_{OXA} and *cfxA6* to beta-lactams, *erm*(F) to macrolide and streptogramins. During spring and summer bla_{OXA}, *ant(3'')-Ia* and *aph(3")-Ib* (conferring resistance to

aminoglycoside), *msr*(E) (macrolide and streptogramins), *arr* (rifampin), predominated, while fall showed a similar profile with the additional presence of tetracycline-resistant genes.
ARGs were similar across spring, summer, and fall, but winter showed a distinct pattern with more tetracycline resistance genes. The remaining ~ 50% of RA in each season is composed by a mean of 525 ARGs. ARGs were categorized by RA: *major* (>1%); *minor* (0.1%-1%); *ultra-minor* (<0.1%). Overall, among the *major* ARGs, only 2 exceeded 10% of relative abundance: *ant(3'')-Ia* at 13% and *blaOXA* at 11.6%; the others ranged between 6.8% and 1.2%. In addition, several *minor* ARGs conferring resistance to carbapenems, vancomycin, methicillin and colistin were detected.
Discussion and Conclusions This study highlights the value of comprehensive multi-target sequencing approach, for a more accurate view of wastewater ARGs compared to methods targeting single genes or limited gene sets. The panel, originally designed for clinical applications, is in this study early applicated in an environmental context. Future optimization could involve targeted panels focused on key ARGs for improved cost-effectiveness and routine monitoring efficiency. Research supported by EU funding within the Next Generation EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, PE13 INF-ACT).

126 - Integrating Phenotypic and Metabolic Characterization to Assess the Effect of Potential Probiotic Candidates on Gut Microbiota Composition, Functionality, and Immune Modulation

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Integrating Phenotypic and Metabolic Characterization to Assess the Effect of Potential Probiotic Candidates on Gut Microbiota Composition, Functionality, and Immune Modulation

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Introduction

Probiotics have long been recognized for their positive impact on gut health, with their ability to restore balance in the microbiota, improve digestion, and support immune function. Their effectiveness is enhanced in multi-strain formulations, where diverse bacteria and yeasts cooperate synergistically. Probiotics are typically isolated from human gut samples, while growing research highlights fermented foods as promising alternative sources for novel

probiotic candidates. This study aims to evaluate the probiotic potential of various lactic acid bacteria (LAB) and yeast strains isolated from different food sources through the characterization of their metabolic and functional traits.

Materials and Methods

An initial screening of approximately 100 LAB and yeast strains was performed to assess their survival under gastrointestinal conditions. Strains exhibiting high resistance were further characterized for their ability to produce exopolysaccharides, as well as for their hydrophobicity and auto-aggregation capacity. Based on our-multi-step and cumulative scoring-based approach, five most promising strains will be characterized for their phenotypic fingerprints through Omnilog Phenotype MicroArray Technology using Prebiome plates, to better understand their metabolic responses to various prebiotic substrates. In the final phase, the best-performing strain will be *in-vitro* evaluated using the Simulator of Human Intestinal Microbial Ecosystem (SHIME) and human intestinal epithelial cell lines to further investigate their impact on gut microbiota composition and functionality.

Results

After exposure to gastrointestinal conditions, LAB strains showed different survival rates, with some not surviving and other maintaining up to 7.02 ± 0.04 Log CFU/mL. Yeast strains had cell densities ranging from 4.95 ± 0.04 to 6.21 ± 0.07 Log CFU/mL. Notably, seven LAB and sixteen yeasts retained high viability (ca. 7 Log CFU/mL and ca. 6 Log CFU/mL, respectively) and were selected for further characterization as potential probiotics candidates. Among them, nine strains were classified as moderately hydrophilic (35 - 70%), while *Hanseniaspora pseudoguilliermondii* YANA2 exhibited high hydrophobicity (72.66% ± 7.50%). After 24 hours, auto-aggregation capacity varied between 27.75% ± 4.28 and 97.66% ± 0.33, with *Pichia kudriavzevii* YKF8 reaching the highest value among all strains.

Discussion and Conclusions

This multi-tiered approach integrates physiological, phenotypic, and metabolic analyses to identify robust potential probiotic candidates with enhanced gastrointestinal survival and functional attributes. The findings of this study will contribute to the development of next-generation probiotics with improved efficacy for gut health applications.

127 - CO-ENTRAPMENT OF COLISTIN AND AURANOFIN INTO HYALURONIC ACID AGAINST MULTIDRUG-RESISTANT BACTERIA ISOLATED FROM WOUND INFECTIONS

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CO-ENTRAPMENT OF COLISTIN AND AURANOFIN INTO HYALURONIC ACID AGAINST MULTIDRUG-RESISTANT BACTERIA ISOLATED FROM WOUND INFECTIONS

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Introduction. The development of new antimicrobial agents is costly and challenging, as reflected in the limited number of approvals in recent years. Promising strategy could be the innovative system of drug delivery and restoring the antibiotics susceptibility through the co-administration of antibiotics and repurposed drugs used as adjuvants. Hyaluronic Acid (HA) is a linear polymer constituted by β-1,4-D-glucuronic acid and β-1,3-*N*-acetylglucosamine units, which confer good biocompatibility, low immunogenicity, high safety and stability. It can act as smart technology for controlling drug release. Auranofin (AUR) has been approved as anti-rheumatic drug, and it is mainly effective against Gram-positive bacteria. The combination of AUR with colistin (COL), an antibiotic used for MDR Gram-negative infections, showed an extended antibacterial spectrum both against Gram-positive and Gram-negative, including COL-resistant strains. Here, the antibacterial activity of the system based on HA-(COL:AUR) against multidrug-resistant (MDR) bacteria isolated from chronic wounds was evaluated. **Materials and Methods**. The system, named S15b, made of COL/AUR/HA in the ratio 1.5/16/1.5 was prepared using the solvent mixture H_2O/ (DMSO/PEG400/Ethanol)/H_2O in the ratio 5:90:5. Antibacterial activity of COL, AUR and S15b was determined by broth microdilution method against MDR clinical isolates and standard reference strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*. The strain *E. coli* DSM 105182, that harbors the plasmid-related *mcr-1* COL-resistance gene, was used for the time-killing assay. Furthermore,

the biofilm-eradicating capability was assessed spectrophotometrically using the colorimetric and metabolic methods, by safranin and XTT assay, respectively. **Results**. The S15b appeared dense and milky, and showed a higher antimicrobial activity against tested strains, in respect to each drug used alone. The highest inhibitory activity against *E. coli* DSM 105182, was detected with 1 microg/mL COL and 16 microg/mL AUR. Furthermore, reduction of more than three orders of magnitude in terms of CFU/mL counts compared to the single drugs and untreated controls as well as a dose-dependent reduction in biofilm biomass and metabolic activity were detected. **Discussion and Conclusion.** These data suggest that the novel S15b formulation may offer a promising strategy to enhance COL efficacy for the treatment of MDR wound infections-associated strains. **Acknowledgments**. This work has been funded by European Union (NextGeneration EU), through the MUR-PNRR project PE_00000007 - One Health Basic and Translational Research Actions-INF-ACT Foundation-Project BAC2_AntiTOP.

129 - Investigation of the knowledge, attitudes, and practices regarding disinfectant use among Sapienza University students

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Investigation of the knowledge, attitudes, and practices regarding disinfectant use among Sapienza University students

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Introduction. Biocides play a key role in protecting human and animal health, with disinfectants representing the most widely used subgroup. However, inappropriate use may pose health risks and contribute to antimicrobial resistance. Despite Article 65 of Regulation (EU) No. 528/2012 encouraging public education on biocide use, recent studies, particularly in the post-COVID-19 context, highlight persistently low levels of knowledge and unsafe practices. Yet, there is a lack of data specific to the Italian population. To address this gap, the present study developed and administered a Knowledge-Attitude-Practice (KAP) questionnaire among students enrolled in various faculties at Sapienza University of Rome.

Materials and Methods. A cross-sectional study was carried out using a web-based survey disseminated via Google Forms, in April 2025, to a convenience sample of Sapienza University

students. The study quantitatively assessed knowledge, attitudes, and behaviors regarding disinfectant use. A binary value was assigned to a total of 26 items and divided in three sections of the KAP survey. Descriptive statistics were used to summarize sample characteristics and responses.

Results. A total of 281 students consented to participate in the study. The majority were under 25 years of age (95.7%), female (70.4%), unmarried (87.9%), and not employed (84.0%). Most participants resided in Rome or its province (66.9%), lived with their family of origin (64.8%), and were enrolled in scientific degree programs (91.1%). Almost half of the participants (45.9%) reported being actively involved in household cleaning routines. The mean rate of positive answers in the knowledge section was 5.3 (±1.8) out of a maximum of 10. For the attitude section, the mean rate was 6.5 (±2.3) out of 10. In the practice section, the mean rate was 2.8 (±1.3) out of 6. Only 29.2% of respondents reported using reliable sources to seek information on the topic, while 27.1% declared they did not use any sources at all. Furthermore, 92.5% felt that the COVID-19 pandemic had, either partially or entirely, influenced their use of disinfectants. When asked to self-assess their knowledge about disinfectant use, 53.7% rated it as poor, 44.1% as sufficient, and only 2.1% considered their knowledge to be optimal.

Discussion and Conclusion. The findings provide a preliminary insight into disinfectant-related behaviors among Sapienza University students, highlighting areas for potential improvement, especially in practical application and source reliability.

132 - Isolation and genomic characterization of vancomycin-resistant Enterococcus faecium from wastewater influents and effluents

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Isolation and genomic characterization of vancomycin-resistant *Enterococcus faecium* from wastewater influents and effluents

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Introduction Antimicrobial resistance (AMR) is a global health threat affecting humans, animals, and the environment. Wastewater and surface waters act as reservoirs and pathways for resistant bacteria, including vancomycin-resistant enterococci (VRE). Among VRE, particular attention is given to *Enterococcus faecium*, an ESKAPE pathogen of clinical concern, with a high capacity for environmental persistence. Monitoring AMR bacteria, including VRE, in environmental waters offers valuable insights into wastewater treatment efficiency and potential risks linked to water reuse. In this study, we investigated the presence, the abundancy, and genomic characteristics of vancomycin-resistant *E. faecium* (VREfm) isolated from influents and effluents of wastewater treatment plants, using culture-based methods and whole-genome sequencing (WGS), and to evaluate wastewater treatment efficiency.
Material and Methods Influent and effluent samples were collected from six wastewater treatment plants. To enumerate VRE isolates, samples were filtered and incubated on Slanetz & Bartley selective agar supplemented with vancomycin (4 µg/mL). Typical colonies were confirmed on EIA medium and by Kirby-Bauer method using vancomycin (5 µg); 33 VREfm isolates were selected on ChromID VRE medium and submitted to WGS using the Illumina technology for MLST and resistome analysis.
Results VRE were consistently isolated from all influent samples (mean concentration: 10^4 CFU/100 mL) and nearly always detected in effluent samples (0-10^2 CFU/100 mL). Total enterococci count averaged 10^6 CFU/100 mL in influents and ranged from 10^2 to 10^3 CFU/100 mL in effluents. Preliminary WGS analysis assigned the 33 VREfm to ST80 (51.5%) or its variant (39.4%) and to ST612 (9.1%). All VREfm strains carried the *vanA* gene, whereas only 1 isolate also harbored the *vanB* gene, both conferring resistance to vancomycin. In addition, all VREfm carried the genes conferring resistance to aminoglycosides [*aac(6')-Ii*] and to macrolides/streptogramins [*msr*(C)]. **Discussion and Conclusions** The detection of VRE in

both influent and effluent samples highlights the incomplete removal of these resistant bacteria by current wastewater treatment processes. Total enterococci count further demonstrate the persistence of these bacteria through the treatment chain, regardless of their resistance profiles. The predominance of the *vanA* gene among VREfm points to the circulation of highly resistant strains in environmental waters. These findings emphasize the urgent need for optimized wastewater treatment strategies to mitigate the environmental spread of clinically relevant antimicrobial resistance pathogens and highlight the importance of integrating environmental monitoring within a broader One Health approach to address global health risks.

133 - Meta-Analysis on the Impact of Probiotic Administration Routes and Subsequent Vaginal Colonization in the Treatment of Bacterial Vaginosis

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Meta-Analysis on the Impact of Probiotic Administration Routes and Subsequent Vaginal Colonization in the Treatment of Bacterial Vaginosis

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Introduction: Bacterial vaginosis (BV) represents the most prevalent form of vaginal dysbiosis among women of reproductive age, with a notable tendency for recurrence and an urgent need for more effective therapeutic strategies. A meta-analysis was conducted to evaluate the impact of probiotic administration routes on BV treatment outcomes, vaginal health, and microbial recolonization. Materials and Methods: Three researchers independently conducted a systematic literature search up to 30 January 2025, using PubMed and Web of Science databases. The following search terms were used: ("probiotics"[All Fields] OR "probiotic"[All Fields]) AND ("bacterial vaginosis"[MeSH Terms] OR "bacterial vaginosis"[All Fields] OR ("vaginal"[All Fields] AND "dysbiosis"[All Fields])). The search was restricted to articles published in English and classified as randomized clinical trials. A total of 987 records were identified. After removing duplicates using EndNote 20, 90 studies were selected based on predefined inclusion and exclusion criteria. Studies were included if they evaluated probiotic treatments administered orally or vaginally, and reported outcomes related to BV resolution, recurrence prevention, improvement of vaginal health markers (such as pH and Nugent score), or post-treatment vaginal colonization. Results: The preliminary analysis indicated that vaginal probiotic administration is significantly more effective in restoring a eubiotic vaginal ecosystem, with higher rates of durable colonization by probiotic strains, mainly *Lactobacillus crispatus, L. jensenii,* and *L. gasseri*, compared to oral administration. However, oral administration demonstrated systemic benefits and greater patient compliance. Discussion and Conclusions: Our findings provided a foundation for optimizing probiotic therapeutic protocols and personalizing strategies for vaginal health management. This meta-analysis underscores the importance of considering the administration route when designing probiotic-based interventions for BV. Ongoing analyses will aim to better define the relationship between specific strains, patterns of recolonization, and long-term clinical efficacy.

Keywords: Bacterial vaginosis (BV), Probiotics, Vaginal Health, Dysbiosis, *L. crispatus, L. jensenii, L. gasseri*, Meta-analysis

134 - Biofunctional Semi-IPN Hydrogels for Antibacterial and Antioxidant Drug Delivery Applications

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Biofunctional Semi-IPN Hydrogels for Antibacterial and Antioxidant Drug Delivery Applications

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Introduction: Biopolymer-based hydrogels are increasingly explored as advanced drug delivery platforms, due to their biocompatibility, mucoadhesiveness, and structural tunability. In this study, a series of semi-interpenetrating polymer network (semi-IPN) hydrogels were developed by combining sodium alginate and chitosan, the latter chemically functionalized with bioactive moieties including caffeic acid (CA) and gallic acid (GA). These modifications were designed to enhance the antioxidant and antimicrobial properties of the hydrogel matrix and to improve drug loading and release performance. Materials and Methods: The hydrogels were loaded with the fluoroquinolone antibiotic lomefloxacin and characterized through physicochemical (FTIR, SEM, swelling index) and biological (DPPH scavenging, antibacterial) analyses. Antibacterial activity was assessed by agar diffusion assay against *Staphylococcus aureus* ATCC 25213. Mueller-Hinton agar plates were inoculated with bacterial suspensions, and hydrogel disks were applied onto the surface. Plates were incubated for 24 hours at 37°C, and inhibition zones were measured in millimeters to evaluate antibacterial efficacy. Results: The structural characterization confirmed successful grafting and the formation of a highly porous matrix in functionalized gels. Drug release assays revealed that the functionalized semi-IPNs modulate release kinetics in a pH- and ionic-strength-dependent manner. Moreover, antibacterial activity against *S. aureus* demonstrated that lomefloxacin-loaded hydrogels exhibited pronounced inhibition, with ALG-CS/LOME, ALG-CS_GA/LOME and ALG-CS_CA/LOME formulations achieving the highest efficacy. The antioxidant assays confirmed that GA and CA grafting significantly improved radical scavenging capacity, further supporting the multifunctionality of the system. Discussion and Conclusions: Beyond their intrinsic antimicrobial activity, the semi-IPN hydrogels present ideal features for localized drug delivery: ease of formulation,

prolonged drug retention, and tunable degradation properties. These findings support their potential application in mucosal therapies. Overall, the combination of mechanical integrity, bioactivity, and controlled release behavior positions these semi-IPN hydrogels as promising candidates for future drug delivery systems targeting infection-prone or oxidative-stress-sensitive environments.

Keywords: Biopolymer-based hydrogels, caffeic acid (CA), gallic acid (GA), antioxidant properties, antibacterial activity, *Staphylococcus aureus*

146 - Genomic epidemiology of listeriosis in northern Italy over a four-year period

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Genomic epidemiology of listeriosis in northern Italy over a four-year period

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Introduction. Listeriosis is the disease with the highest case fatality and hospitalisation rates in EU countries along with West Nile virus infections, according to the last report by the EFSA and the ECDC. Due to its impact on public health and the potential for foodborne outbreaks, listeriosis is a priority disease to be subjected to whole-genome sequencing (WGS)-enhanced surveillance.
Here, we present epidemiological and laboratory surveillance data of listeriosis cases occurred in Lombardy, northern Italy, over a four-year period (2021–2024).

Materials and Methods. Clinical microbiology laboratories of Lombardy sent *Listeria monocytogenes (Lm)* isolates obtained from the biological samples of hospitalised patients with listeriosis to the regional reference laboratory at the Università degli Studi di Milano. Demographic and clinical data were collected anonymously from standardised report forms accompanying *Lm* isolates. WGS was performed through Illumina technology. Cluster analysis was conducted with core-genome multi-locus sequence typing (cgMLST) and single nucleotide polymorphisms (SNPs) approaches. We combined laboratory data of human isolates with those of food collected as part of outbreak investigations.

Results. We identified 271 cases of listeriosis (median age: 73 years, range 0–101), presenting in the 80.8% as primary sepsis. The mean annual case fatality rate was 17.1%. Fourteen cases (5.2%) were maternal-neonatal. *Lm* strains belonged to 42 sequence types (STs), including a newly identified one (ST3287). The predominant ST was ST155 (50 isolates). Overall, cgMLST- and SNPs-based analyses showed the presence of 35 clusters comprising 144 isolates (53.1%), with the largest caused by a ST155-clone (37 cases). Epidemiological investigations revealed that this cluster was part of an Italian outbreak associated with frankfurter consumption. Seventeen clusters included also isolates from the food chain. Overall, Lombardy was involved in 8 strong-evidence outbreaks at national or EU scale, whose sources were meat, fish, and vegetable products.

Discussion and conclusions. The annual incidence of invasive listeriosis in Lombardy (0.66/100,000) was higher than those reported by the ECDC in Italy (0.48/100,000) and the EU average (0.61/100,000). Most cases were observed in the elderly, as in other EU countries. This might be explained by EU's ageing population and the increasing prevalence of chronic age-related diseases. Our findings highlight the importance of a One Health approach to public health surveillance of listeriosis. Indeed, monitoring of whole-genome sequences from human and food isolates, complemented with epidemiological data, allowed us to detect several outbreaks and possible sources of infection.

154 - Structure-Based Design of α-Helical Antimicrobial Peptides via Main Mechanical Forces (MMFs) as Dual-Action Agents Against Multidrug-Resistant Bacteria

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Structure-Based Design of α-Helical Antimicrobial Peptides via Main Mechanical Forces (MMFs) as Dual-Action Agents Against Multidrug-Resistant Bacteria

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Background: The growing threat of multidrug-resistant (MDR) bacterial infections has driven the search for innovative therapeutic strategies beyond traditional antibiotics. Antimicrobial peptides (AMPs), especially those with α-helical conformations, are considered promising alternatives due to their rapid membrane-disruptive action and reduced tendency to induce resistance. However, naturally occurring AMPs often suffer from limitations such as poor stability and high production costs. This study presents a new class of synthetic α-helical peptides rationally designed using the Main Mechanical Forces (MMFs) methodology. This structure-based approach allows precise control of peptide conformation through calculation of dihedral angles, optimizing interactions between amino acid side chains to stabilize amphipathic helices. The designed peptides incorporate two synergistic antimicrobial features: (i) a spatially segregated distribution of cationic and hydrophobic residues to facilitate bacterial membrane disruption, and (ii) a chelating moiety to sequester essential metal ions, impairing bacterial metabolism and enhancing bactericidal activity. Materials and methods: The antibacterial activity of HT2, its analogs K1 and K1-4-5, and their combinations with the chelating agent allomaltol was evaluated using the broth microdilution method against MRSA (*S. aureus* USA300), *K. pneumoniae* ATCC 700603, and *E. coli* ATCC 25922. Peptides were tested at concentrations ranging from 600 to 9.38 μM. MICs were determined after overnight incubation at 37 °C. Results: HT2 exhibited strong activity against MRSA (MIC = 18.75 μM),

moderate against *E. coli* (75 μM), and limited against *K. pneumoniae* (150 μM). K1 showed no activity, while K1-4-5 retained partial efficacy. Remarkably, co-administration of K1-4-5 with allomaltol enhanced potency, with MICs of 37.5 μM against MRSA and *E. coli,* and 75 μM against *K. pneumoniae*, suggesting a synergistic effect. Discussion and Conclusions: These findings validate the MMFs-guided design as an effective strategy for generating stable, multifunctional α-helical peptides with therapeutic potential. The approach supports the development of a new class of AMPs capable of addressing the urgent need for broad-spectrum, resistance-resilient antimicrobials.

Keywords: Bacteria Multidrug-Resistant (MDR), Antimicrobial peptides (AMPs), Main Mechanical Forces (MMFs), Minimum Inhibitory Concentrations (MICs)

168 - Effect of fermentation media on Bifidobacterium longum DSM 32947 features

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Effect of fermentation media on *Bifidobacterium longum* DSM 32947 features

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INTRODUCTION Several studies have shown that bifidobacteria are among the first colonizers of the human gut and are essential for proper immune system development. After weaning, the intake of solid foods, containing more complex substrates, leads to a shift in the gut microbiota composition and the relative abundance of bifidobacteria decreases. Due to their health-promoting properties, various probiotic strains of bifidobacteria have already been commercialized. In this work, we have investigated a new promising strain, namely *Bifidobacterium longum* subsp. *longum* DSM 32947, focusing on how its biological properties can be affected by the cultivation media.
MATERIALS AND METHODS *B. longum* was cultivated in industrial media in 1L bioreactors. The composition of the growth media was adjusted to obtain four different combinations using two carbon sources, glucose and lactose, and two nitrogen sources, yeast extract and casein peptone. For each combination the fermentation yield was evaluated, the resulting biomass was freeze-dried and stability studies were carried out to evaluate the bacterial viability over time. Moreover, the antimicrobial activity of the Cell Free Supernatant (CFS), isolated from each fermentation, was tested against *S. aureus*, *P. aeruginosa* and *E. coli* reference strains via microdilution method. Each fermentation was also characterized in terms of membrane vesicle production and proteomic analysis was performed on the freeze-dried cells.
RESULTS No significative differences in terms of fermentation yields was detected between the four different combinations, but it was observed that the casein-glucose combination was the best in terms of stability over time. The CFS isolated from the fermentations showed similar MIC and MBC values against all pathogens tested, except for casein-glucose and yeast extract-glucose that showed an MIC of 30µl/100µl for *E. coli*. The casein-glucose combination resulted in a lower production of vesicles, corresponding to 10^{11} MVs/ml, while the other combinations showed 10^{13} MVs/ml.

DISCUSSION AND CONCLUSIONS Media formulation in the production of probiotics is essential to optimize the fermentation yield as well as the final product in terms of stability and bioactivity; the composition of the substrate has an impact on bacterial metabolism, hence on bacterial byproducts correlating to specific outcomes, such as antimicrobial activity. These (preliminary) data could be a starting point to understand how different carbon and nitrogen sources influence the properties of *B. longum* DSM 32947 and what can be improved to obtain a more efficient strain in terms of health-promoting activity. Undoubtedly, future investigations, partially ongoing, are needed to deepen the understanding of this bacterial strain.

169 - Exploring molecular mechanisms of anti-HSV-1 antiviral activity of a Sicilian Almond Skin Extract

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Exploring molecular mechanisms of anti-HSV-1 antiviral activity of a Sicilian Almond Skin Extract

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Introduction. In recent years, almond fruit and its by-products have been widely studied for their biological activities. Particularly, almond skin extracts (ASE) have been shown to block the diffusion of HSV-1 particles to other nearby cells, in a permissive cellular experimental model. However, the mechanisms involved have not clearly elucidated. In the present study, the antiviral effects of ASE obtained from the Sicilian cultivar "Fascionello" were investigated to discern the underlying molecular mechanisms. **Materials and Methods.** ASE was obtained through a manual stripping process previously fine-tuned at the University of Messina. Semi-permissive human U937 monocytic cells, derivative U937-IkB-alpha-DN cells with the murine dominant negative IkB-alpha sequence, generated in our laboratory, and Vero cells, were used. Commercial murine monoclonal antibody against HSV-1 gD, FITC-conjugated anti-mouse IgG antibody, anti-HVEM monoclonal antibody, HRP-conjugated anti-mouse IgG antibody, and the murine anti-beta-actin monoclonal antibody, were utilized. The HSV-1 'F' strain was used for infection. ASE was dissolved in DMSO, diluted in fresh medium and the solution was sterilized by filtration. In some experiments ASE was added to U937 cells at the end of the HSV-1 adsorption phase, while in others, infection was synchronized on ice at 4°C. Autophagy was analyzed using the Abcam's Autophagy Assay Kit. Apoptosis was analyzed with the Hoechst nucleic acid probe and the fluorogenic reagent "CellEvent™ Caspase-3/7 Green Detection Reagent, for the detection of caspase 3 and 7 activation. Level of viral DNA was quantified by real-time PCR. U937 cells subjected to synchronized infection were analyzed for the expression of the HVEM receptor and the viral glycoprotein gD, by western blotting. **Results.** The multifunctional experimental approach adopted to investigate the antiviral effects of ASE as a function of dose and time to identify the phases and molecular mechanisms through which this almond bio-product interferes with HSV-1 life cycle, revealed that ASE pretreatment effectively inhibits HSV-1 replication in U937 cells by interfering with the viral entry phase. This effect very likely involves modulation of the host cell receptor HVEM, critical for membrane fusion and viral penetration. **Discussion and Conclusions.** The results of this study provide robust evidence supporting and explaining the antiviral properties of ASE. When optimally stored to preserve its bioactive properties, this byproduct can serve as a valuable resource for pharmaceuticals. Moreover, valorizing Avola almond by-products promotes a circular economy model,

facilitating optimal management of the agri-food industry waste and contributing to environmental sustainability.

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170 - A One Health Perspective on Carbapenem-Resistant Pseudomonas aeruginosa (SAMPAN study)

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A One Health Perspective on Carbapenem-Resistant *Pseudomonas aeruginosa* (SAMPAN study)

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Introduction

Antimicrobial resistance (AMR) is a growing global threat, potentially causing up to 10 million deaths annually by 2050. *Pseudomonas aeruginosa*, especially in its carbapenem-resistant form (CRPA), is listed among WHO's critical priority pathogens. Due to its ubiquity in moist environments, CRPA requires integrated One Health surveillance. The present abstract shows the italian results of the the multicenter SAMPAN study (Italy, Netherlands, Indonesia) which aim is to develop a global surveillance strategy by exploring CRPA transmission routes and environmental and human reservoirs.

Materials and Methods

From 2022 to 2024, healthy volunteers from high-risk areas and hospitalized patients (within 48h of admission) were recruited. Swabs (pharyngeal, umbilical, perineal) were cultured on M-PA-C agar and identified using MALDI-TOF. Antimicrobial susceptibility was assessed using Vitek2. Environmental sampling included: a) hospital water inlets and hospital wastewater treatment plants (hWWTP); b) influents and effluents from municipal WWTPs (mWWTP); c) river sections upstream and downstream of discharge points. Water samples were filtered and incubated in Asparagine Proline Broth with vancomycin. Genomic sequencing of CRPA isolates is in progress.

Results

Among 250 healthy volunteers, 5.6% tested positive for susceptible *P. aeruginosa*; no CRPA was detected. Among 340 hospitalized patients, 7.1% were colonized with *P. aeruginosa*, and 0.6% with CRPA. CRPA was found in 80% of hospital wastewater samples in Rome. In the community, 43% of municipal WWTP influents and 57% of effluents tested positive for CRPA. River samples upstream and downstream of discharge points were positive in 14% and 43% of cases, respectively. Despite treatment, CRPA persisted in effluents and surrounding waters.

Discussion and Conclusions

Despite the diffusion of CRPA in community water samples the prevalence of CRPA colonization remains law in the city of Rome. SAMPAN will enable analysis of CRPA transmission in three countries with differing prevalence, climates, and water infrastructures. Combining clinical, environmental, and genomic data allowed the identification of risk factors, reservoirs, and resistance genes. The findings will support the development of a globally applicable, smart surveillance strategy and inform measures to prevent further CRPA spread.

184 - Antagonistic Activity of Commensal-Derived Molecules Against Helicobacter pylori

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Antagonistic Activity of Commensal-Derived Molecules Against *Helicobacter pylori*

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Introduction. The gastrointestinal tract hosts a complex microbial ecosystem contributing to host defences through structural and metabolic functions. Antagonistic interactions between commensals and pathogens have emerged as promising strategies for infection control. Increasing evidence indicates that commensal bacteria can secrete bioactive factors able to modulate the microbial milieu and suppressing pathogen growth. These molecules may interfere with colonization, virulence, and persistence of pathogens such as *Helicobacter*

pylori, a key gastric pathogen linked to gastritis, ulcers, and gastric cancer. This study aimed to evaluate the *in vitro* antagonistic potential of secreted molecules by selected commensal *Neisseria* species against *H. pylori*. **Materials and Methods.** Three *H. pylori* strains (ATCC 43629, 2A12, 13A13) were cultured under microaerophilic conditions. Commensal *Neisseria* strains (*N. flavescens*, *N. lactamica*, *N. cinerea*, *N. macacae*), isolated from healthy individuals, were grown aerobically. An overlay assay was developed using Columbia Blood Agar. The bottom layer was supplemented with live *Neisseria* cells/filtered or ultracentrifugated culture supernatants, the top layer supported *H. pylori* growth. After incubation, the ratio between the *Neisseria*-containing plates and control plates' Optical density (OD_{600nm}) was determined. Moreover, *N. macacae* ultracentrifugated supernatant was tested in co-culture assay: *H. pylori* ATCC was resuspended in Brucella Broth, treated with 2% v/v *N. macacae* ultracentrifugated supernatant, and incubated under microaerophilic conditions. Viability was measured at 24, 48, and 72 h. **Results.** All *Neisseria* strains except *N. flavescens* inhibited *H. pylori* to varying degrees. *N. macacae* showed the strongest inhibition across all *H. pylori* strains. Overlay assays using both the pellet and filtered supernatant of *N. macacae* showed comparable inhibition, highlighting the potential of secreted factors. In addition, preliminary broth inhibition results further demonstrated a marked reduction in the growth of *H. pylori* ATCC upon treatment with *N. macacae* ultracentrifugated supernatant, strongly supporting its antagonistic effect. **Discussion and Conclusions.** *N. macacae* acts as a potent *in vitro* inhibitor of *H. pylori*. Its ultracentrifugated supernatant showed strong inhibitory effects, suggesting involvement of secreted molecules such as Outer Membrane Vescicles, bacteriocins, or small effectors. These findings support the potential of certain commensal *Neisseria* strains as modulators of gastric pathogen fitness. Future studies will aim to characterize the active components and assess their roles in host-pathogen interactions.

***188 - Resistant or just persistent? evaluating the potential for phenotypic reversion in antibiotic-free environments*.**

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Resistant or just persistent? evaluating the potential for phenotypic reversion in antibiotic-free environments

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Introduction: Antimicrobial resistance (AMR) is a growing concern in both human and veterinary medicine. While resistant bacterial strains gain a selective advantage in environments with antimicrobial exposure, resistance-conferring mutations may come with fitness costs, such as impaired protein synthesis or reduced growth rates. In theory, under non-selective conditions, susceptible wild-type subpopulations may outcompete resistant ones over time. This study explored whether prolonged propagation in antibiotic-free broth could lead to a phenotypic shift from resistance to susceptibility in macrolide-resistant bacterial strains, using *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) as models. **Materials and Methods:** Two MG and three MS strains with high-level resistance to erythromycin and variable susceptibility to other macrolides were selected from the strain collection of the Istituto Zooprofilattico Sperimentale delle Venezie. Serial passages were performed in Avian Mycoplasma Experience broth, both without antibiotics. MG strains underwent 100 passages, while MS strains were propagated up to 40 passages. At specific timepoints, broth cultures were plated on agar with or without macrolides at different concentrations to assess potential phenotypic reversion. Colony counts were normalized to viable bacterial titers to ensure comparability. **Results:** Throughout the 100 serial passages in antibiotic-free broth, MG strains showed no appreciable reduction in resistance when plated on agar containing erythromycin or tylosin. Colony counts on macrolide-containing plates remained consistent with those on antibiotic-free agar at both the first and hundredth passages, indicating that resistant populations persisted over time. Similar trends were observed in MS strains after 40 passages. **Discussion and Conclusions**: The results suggest that, despite the absence of selective pressure, macrolide-resistant MG and MS strains maintain phenotypic stability over extended

propagation. This indicates that the hypothesized fitness cost associated with macrolide resistance may not be sufficient to allow susceptible subpopulations to outcompete resistant ones under the experimental conditions. Moreover, no reversion to susceptibility was observed. However, it cannot be excluded that periods without antimicrobial pressure longer than those tested may eventually affect the phenotype of these microorganisms. As a next step, whole genome sequencing will be used to investigate whether genetic variations at known resistance-associated loci happened during serial passages—specifically to assess whether susceptible (non-mutant) subpopulations increased in number without fully outcompeting the resistant strains, to an extent not detectable through phenotypic assays alone.

199 - Comparative Metagenomic Analysis of Urban and Hospital Wastewaters Using Nanopore Sequencing

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Introduction

The rapid spread of antimicrobial resistance (AMR) in humans, animals and environment poses growing challenges to global health. Among environmental sources, urban and hospital wastewaters are recognised as the primary sources of environmental contamination by AMR. However, high-resolution resistome profiling of hospital wastewater remains limited in many countries and specifically in Africa.

This study aimed to investigate differences in microbial communities and resistome composition between hospital and urban wastewater in Conakry Guinea using a shotgun

metagenomic approach based on Nanopore sequencing, to prioritise risk sources and inform targeted strategies for AMR mitigation.

Materials and Methods

Wastewater samples from an urban area (n = 4) and a hospital (n = 4) in Guinea were grouped by origin and time. DNA was extracted from 20 mL of filtered (0.22 µm) wastewater using the PowerWater DNA kit (Qiagen), quantified with the NanoDrop spectrophotometer (TermoScientific), and prepared using the Rapid Barcoding Kit (Oxford Nanopore). Sequencing was performed on a MinION MK1D device.

Microbial communities and resistomes were analyzed through bioinformatic pipelines, with statistical significance assessed using the ANOSIM test and the PERMANOVA test, respectively. Replicates differences were tested with the t or Wilcoxon tests based on Shapiro-Wilk normality results.

Results

Microbial communities differed significantly between urban and hospital wastewater ($p = 0.029$), driven by the higher relative abundance of *Enterobacteriaceae* (hospital) and *Campylobacteriaceae* (city). No significant differences emerged in the overall resistome composition. However, specific resistance gene classes showed distinct patterns: beta-lactam resistance and efflux pump genes were more abundant in *Enterobacteriaceae*, *Pseudomonadaceae*, and *Staphylococcaceae* in hospital samples, while tetracycline resistance genes were more prevalent in *Bacteroidaceae* in urban samples. Overall resistance levels (expressed as genomic copies per gigabase) were exceptionally high, compared to works already published in literature.

Discussion and Conclusions

This study reveals differences in the composition of bacterial communities between hospital and urban wastewater in Guinea, despite similar resistomes. The elevated abundance of clinically relevant resistance genes in hospital-associated bacterial families underscores the role of hospital effluents as key hotspots for AMR dissemination. Meanwhile, the high levels of resistance genes also found in urban wastewater may suggest a widespread community burden. These findings support the integration of wastewater surveillance into national AMR monitoring and the need for targeted interventions in both healthcare and community settings.

200 - Unraveling the microbial and immune landscape of Prostate Cancer: toward novel diagnostic biomarkers

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Unraveling the microbial and immune landscape of Prostate Cancer: toward novel diagnostic biomarkers

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Introduction Prostate cancer is the most common malignancy among men and the fifth leading cause of cancer-related death globally. Despite its prevalence, early diagnosis remains challenging due to the low tumour specificity of the current biomarker, prostate-specific antigen (PSA). This limitation has prompted efforts to identify novel, tumour-specific biomarkers. Emerging evidence highlights the role of chronic inflammation, sexually transmitted infections (STIs) and human endogenous retroviruses (HERVs) in prostate carcinogenesis, offering new perspectives for risk stratification and precision medicine approaches.

Materials and Methods A total of 244 patients with different prostate conditions—prostate cancer (PCa), precancerous lesions (Borderline), and benign prostatic hyperplasia (BPH)—

were enrolled in the study. DNA extracted from urine samples was screened for a panel of STIs using multiplex RT-PCR. Additionally humoral immune responses against *Trichomonas vaginalis*, *Mycoplasma hominis*, as well as HERV-K, HERV-H, and interferon regulatory factor 5 (IRF5) were assessed by ELISA and compared to age-matched healthy controls (HCs).

Results RT-PCR revealed a higher prevalence of STIs in PCa and Borderline groups compared to BPH with *M. hominis* being the most frequently detected pathogen. Seropositivity for anti-*M. hominis* antibodies was significantly higher in Borderline (86.96%), PCa (64.93%), and BPH (50.57%) groups compared to HCs (28.70%). Similarly, anti-*T. vaginalis* antibody levels were increased in Borderline (60.87%), BPH (52.87%), and PCa (51.49%) patients compared to HCs (28.70%), No significant humoral response was detected against HERV-K or HERV-H. Anti-IRF5 antibody levels were significantly reduced in PCa (32.09%), BPH (22.99%), and Borderline (13.04%) patients compared to HCs (67.83%).

Discussion and Conclusions Our findings suggest a multifactorial model for prostate cancer pathogenesis involving chronic infections, immune modulation, and HERVs elements. The elevated prevalence and seropositivity rates for *M. hominis* and *T. vaginalis* in patients with PCa and precancerous lesions points to their potential role in establishing a pro-inflammatory microenvironment that may promote tumour initiation and progression Although no specific humoral response to HERVs was observed, the marked reduction of anti-IRF5 antibodies in patients points to possible immune suppression or dysregulation, consistent with IRF5's putative tumour-suppressor function in prostate cancer. Altogether, these data underscore the importance of host-pathogen interactions and immune regulation in prostate carcinogenesis. Future work should explore the integration of these biomarkers into predictive models for early detection and patient stratification.

209 - Exploring the antimicrobial potential of Compost Tea from Organic Waste

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Exploring the antimicrobial potential of Compost Tea from Organic Waste

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Introduction: The need for innovative, sustainable, and effective antimicrobial strategies has become increasingly urgent due to the rising threat of viral and bacterial outbreaks, as well as the limitations in discovering new drugs. Bio-based solutions derived from organic waste present a compelling avenue for addressing these challenges. Among these, compost tea (CT)—a liquid extract produced through the fermentation of composted organic matter—has emerged as a promising candidate. Traditionally recognized for its applications in agriculture to suppress plant pathogens and promote crop health and resilience, CT also shows promise for broader applications, including the prevention of microbial infections. Its complex composition, including bioactive compounds, micronutrients, and diverse microbial consortia, provides a unique opportunity for exploring its antimicrobial activity against human pathogens. This study analyzed CTs from bell pepper (CT-BP) and citrus (CT-C) composted waste, assessing their antioxidant, antiviral, and antimicrobial activities. **Material and Methods:** Chemico-physical characterization of compost tea was carried out via NMR and Gas Chromatography-Mass spectrometry. Antioxidant activity was assessed using both the ABTS and DPPH assays. Antimicrobial screening of CTs was performed against different bacterial microorganisms through diffusion disk assay (DDK) and broth microdilution method (MIC). Plaque-reduction assays were conducted to explore the CTs antiviral potential. **Results:** NMR spectroscopy and thermochemolysis assessed CT-BP had more oxidized lignin derivatives, while CT-C contained intact lignin structures. The ABTS assay revelead that UV-Vis absorbance inhibition was highest in compost teas derived from bell pepper (64.1%), whereas CT citrus exhibited a significantly lower inhibition value. A similar trend was observed in the DPPH assay,

where CT bell pepper confirmed the greatest scavenging potential due to its major phenolic content. Both CTs effectively inhibited Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), with CT-BP recording significant efficacy. However, Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) were more resistant. CT-BP also demonstrated potent antiviral effects against enveloped viruses like herpes simplex virus type 1 (HSV-1) and respiratory syncytial virus (RSV). **Conclusions and discussions**: The elective efficacy of CTs against bacterial and viral pathogens suggests that their bioactivity is closely linked to their molecular composition, particularly the presence of oxidized lignin fragments and polyphenolic compounds. The lack of activity against Gram-negative bacteria and non-enveloped viruses further indicates that the mechanisms of action are likely dependent on interactions with bacterial cell membranes and viral envelopes. Overall, these findings support compost-derived extracts as sustainable bioactive agents, offering nature-based alternatives to conventional treatments. Further research could enhance their extraction and scalability for biomedical applications, aligning with circular economy principles and green biotechnology.

219 - Antibiotic resistance, biofilm production, and critical antibacterial associations in clinical Staphylococci of veterinary and human origin

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Antibiotic resistance, biofilm production, and critical antibacterial associations in clinical Staphylococci of veterinary and human origin

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Introduction: Staphylococcal infections pose significant challenges in companion animals and humans. This study aimed to: 1- assess resistotypes of *Staphylococcus* spp. against critical antibiotics, 2- evaluate biofilm production, and 3- analyse associations between resistance patterns, biofilm formation, and multidrug resistance (MDR) in a One Health context.

Methods: A total of 2,567 clinical samples from infected dogs, cats, and humans were cultured on Columbia Blood Agar and Chromatic *S. aureus* media. Isolates were identified by MALDI-TOF MS and tested for susceptibility to 15 critical antibiotics (5 HIA, 6 HCIA, 4 HPCIA) using Kirby-Bauer/E-test methods (CLSIVet 2024 - EUCAST 2025). Biofilm production was detected via Congo Red agar. Multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) isolates were defined. Statistical analyses (Chi-Square, Cramer's V, Goodman-Kruskal Lambda) assessed resistance associations, dependence, and predictivity.

Results: Among 230 *Staphylococcus* isolates (*S. pseudintermedius*: 48.3%; *S*. CoN: 33.3%; *S. aureus* subsp. *aureus*: 10%; *S. intermedius*: 6.5%; *S. delphini*: 0.9%; *S. schleiferi* subsp. *coagulans*: 0.9%), all were susceptible only to linezolid. None were PDR while two feline strains (*S. pseudintermedius* and *S. haemolyticus,* 0.9%, 2/230, non-biofilm producers) were XDR. Highest resistances occurred against benzylpenicillin (77.9%), cefoxitin/oxacillin (47.8%, $P<0.0001$), amoxicillin-clavulanate (42.6%, $P<0.0001$), followed by erythromycin (41.7%), clindamycin (41.3%), norfloxacin (37.8%), tetracycline (32.2%), trimethoprim/sulfametoxazole

(27.4%), imipenem (11.7%), and gentamicin (8.7%). Resistances to human antibiotics (HCIA: mupirocin 1.5%, daptomycin 3.1%) were observed for canine staphylococci. Resistances *versus* HPCIA (vancomycin 3.1%, teicoplanin 5.3%) were observed for strains of animal origin, except one strain. A high positive significant associations were observed between MDR and methicillin resistance, predicting methicillin resistance from MDR and *viceversa* (Cramer's V=0.7109, Lambda=0.6636) and between clindamycin-erythromycin resistance (Cramer's V=0.6259; Lambda=0.7925), predicting resistance to clindamycin from erythromycin and *viceversa* (Lambda=0.785). A low non-significant association was recorded between MDR and slime/biofilm production (Cramer's V=0.1302).

Discussion and Conclusions: This study highlights alarming resistance trends, including to highest-priority antibiotics, in staphylococci from veterinary and human sources. The findings underscore the need for integrated AMR surveillance across species to guide stewardship policies and mitigate One Health threats.

232 - NANOBLOC® Project: Development of an European-centered Antiviral and Antimicrobial Nanocoating Platform

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NANOBLOC® Project: Development of an European-centered Antiviral and Antimicrobial Nanocoating Platform

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Introduction: Bacterial and viral infections severely affect human health, economies and societies, globally. This impact is mainly ascribable to the rapid and easy transmission of pathogens via direct contact with infected individuals or indirect contact with contaminated surfaces. To address this issue, the European Consortium NANOBLOC® was created, establishing an interdisciplinary cocreation alliance among 4 world-class universities and institutes and 5 leading industrial companies, and bringing together high-level expertise in material synthesis, coating processes, microbiology and virology. The goal of the project,

funded by Horizon Europe, is to develop and upscale reliable "*Made in Europe*" antimicrobial and antiviral nanocoatings to minimize the spread of pathogens among the community. Materials and Methods: Eco-sustainable methods were used to synthetize nanocoatings deposited on a variety of substrates: filter materials, textiles and diverse high-traffic solid surfaces. Materials that maintained high functionality after coatings deposition were selected for the study. The assessment of cytocompatibility, ecotoxicity, antimicrobial and antiviral potential of nanocoatings was performed following specific guidelines of the International Organization for Standardization (ISO). Next, antiviral and biocidal mechanisms of action were investigated applying novel protocols for the most active coated substrates. Results: Coatings were synthetized and characterized by their physico-chemical and mechanical features. Notably, four categories of coatings for air filtration systems, with a good functionality and antibacterial action, were endowed also with broad-spectrum antiviral activity against a panel of respiratory viruses, as HCoV-OC43, HRV-A1, IFV-A H3N2, and AdV-5. Of note, direct inactivation of viral particles by coatings represented the main mechanism of action involved. Furthermore, the selected coatings showed a good cytocompatibility profile in vitro and no ecotoxicity was evidenced using the bioluminescent *Vibrio fischeri* model. Discussion and Conclusions: Overall, NANOBLOC® Consortium is contributing to the development of industrially scalable, versatile and green technologies to counteract the transmission of pathogens. Additionally, specific assays for the evaluation of nanocoatings antiviral activity were designed and these protocols have been proposed to the European Committee for Standardization Workshop Agreement (CWA).

238 - A new eco-sustainable strategy to counteract resistant Staphylococcus pseudintermedius and Pseudomonas aeruginosa in the Lubbock Chronic Wound Biofilm model

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A new eco-sustainable strategy to counteract resistant *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* in the Lubbock Chronic Wound Biofilm model

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Introduction: The increase of multi-drug-resistant (MDR) strains represents a global threat reducing the ability to successfully treat microbial infections. In particular, the increasing of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and MDR *Pseudomonas aeruginosa* strains associated with dog wounds represents an important challenge for the treatments. This urgent problem is strongly connected to the dynamic zoonosis which need to be monitored to fight the antimicrobial resistance (AMR) phenomenon by using an eco-sustainable strategy in a "One Health" approach. The aims of this study were: i) to set up a suitable *in vitro* model, the Lubbock Chronic Wound Biofilm (LCWB), to reproduce the dog chronic wound; ii) to suggest an eco-sustainable strategy by using the Light-Emitting-Diodes (LEDs) and the recombinant NK1 protein to counteract the MDR strains colonization in a dog chronic wound.

Materials and Methods: MRSP 115 and MDR *P. aeruginosa* 700 strains, isolated from dog wounds, were used to set up the LCWB at 24, 48 and 72h. The LCWBs were evaluated in terms of volume, weight and microbial CFU/mg. The microbial spatial distribution in the LCWBs was assessed by SEM and CLSM imaging. The antimicrobial/anti-virulence actions of the LEDs and NK1 alone and combined each other were determined against MRSP and *P. aeruginosa* also when they were mixed in the LCWB by microbial CFUs determination, biomass and microscope observations. Results: The best incubation time for the LCWB production in terms of volume (3.38 cm^3 ± 0.13), weight (0.86 gr ± 0.02) and CFU/mg (up to 7.05 x 10^6 CFU/mg ± 2.89 x 10^5) was 48h. The SEM and CLSM images showed a major viable microbial colonization at 48h with a non-mixed bacteria with a prevalence of MRSP on the surface and *P. aeruginosa* in the depth of the wound. When the LEDs were combined with NK1, a relevant antimicrobial action (85.98% of MRSP CFU/ml reduction and 44.29% of *P. aeruginosa* CFU/ml reduction) and significant anti-virulence effects (motility/twitching and biofilm reductions) were obtained. Significant CFU/mg reductions were observed when the LEDs were combined with NK1 on the LCWB. Discussion and Conclusions: The obtained findings demonstrate the capability of *S. pseudintermedius* to grow together *P. aeruginosa* in the LCWB model, representing a suitable model to reproduce the animal chronic wound *in vitro* and the combination of LEDs and NK1 represents an innovative non antibiotic and eco-sustainable strategy to counteract the MDR strains in the dog wound infections.

Funded by the European Union – Next Generation EU, National Recovery and Resilience Plan (PNRR), Mission 4 "Education and research" - Component 2 "From research to business" of the National Recovery and Resilience Plan (PNRR). A heparan sulfate proteoglycan binding protein and Light-Emitting Diodes-LEDs/Complex Electromagnetic Fields-CMFs technologies as innovative eco-sustainable strategies to counteract chronic wound infections associated to *Staphylococcus pseudintermedius* resistant strains: an interdisciplinary approach to animal-human health. P20224AEAC.

240 - Sustainable Technologies for Bio-Char production and its Microbial Bioactivation for Soil Improvement

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Sustainable Technologies for Bio-Char production and its Microbial Bioactivation for Soil Improvement

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1. **Introduction:** The present study aimed to design a sustainable biotechnological process using pistachio by-products (shells and husks) as substrates to produce biochar and bio-activated biochar, which will then be employed as growth factors in agriculture.
2. **Materials and Methods*:*** Biochar from pistachio shells and husks, produced by thermochemical gasification, was divided into four fractions based on its particle size

(200-500 μm, 500-2000 μm, 2000-5000 μm, >5000 μm) and analyzed in terms of chemical-physical parameters (pH, density, electrical conductivity, higher calorific value, humidity, particle size distribution, water retention) and inorganic and organic parameters (C, H, N, O, S, phosphorus, metals, polycyclic aromatic hydrocarbons, dioxins and phuranes). Bioactivation using the four fractions was performed using three bacterial strains, *Streptomyces nigra* (new isolated), *Streptomyces violarus* ATCC 15891 and *Bacillus subtilis* ATCC 6633. A bioassay-based evaluation of biochar toxicity was performed on a A549 cell line.

3. **Results:** Most of the biochar particles had a size between 500 μm (30.8% and 22.5%) and 2.0 (29.9% and 48.5%) mm, with a lower proportion (16.7% and 20.9%) >5.0 mm in the biochar from husks and shells, respectively. Except for calcium, metals were detected in higher concentrations in the biochar from husks. Particle size did not affect cell survival, with no significant differences between the two biochars. Biochar treatment did not affect cellular morphology and monolayer confluence in A549 cells, highlighting no effect associated with the different sizes.
4. **Discussion and Conclusion:** Microbially bioactivated biochar from pistachio by-products bioactivated may serve as a sustainable biofertilizer to boost soil fertility and crop production. This will result in a reduction in the use of agrochemical compounds in agriculture, together with an integrative approach to valorize pistachio by-products within a circular economy goal.

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244 - Effect of New Bromelain-Based Polyenzymatic Complex plus N-Acetylcysteine against Urinary Tract Infections

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Effect of New Bromelain-Based Polyenzymatic Complex plus N-Acetylcysteine against Urinary Tract Infections

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Introduction. Urinary tract infections (UTIs) represent one of the most common bacterial infections with significant morbidity and medical costs together with a negative impact on patient's quality of life in terms of intimate and social relationships. *Escherichia coli* represents one of the most responsible bacteria involved in the UTIs. The UTIs eradication rate is affected by the diffusion of antibiotic-resistant strains and biofilm formation. The aim of this study was to investigate the antimicrobial, anti-motility, and anti-biofilm effects of a novel polyenzymatic complex (DIF17BRO®), a blend of fruit and stem extract of *Ananas comosus* (L.) Merr., in combination with NAC (Formulation) against UTI related *E. coli* strains. **Materials and Methods.** *E. coli* ATCC 10536 and clinical *E. coli* strains were used for the study. The antimicrobial activity of complex DIF17BRO® and NAC was evaluated through MIC and MBC determinations. The anti-motility effect of Formulation (DIF17BRO® + NAC) was tested by assessing swimming/swarming/twitching and the anti-biofilm activity was evaluated through biomass quantification, CFU/mL determination and Live/Dead microscopic observation.

Results. The MICs and MBCs of NAC for all *E. coli* strains were 5 mg/mL and 10 mg/mL, respectively, except for *E. coli* PNT (MBC: 5 mg/mL). Our results demonstrate that the Formulation did not affect the swimming motility, but was able to affect swarming and twitching motility, with a reduction of the swarming motility of *E. coli* ATCC 10536 and a decrease of the normal adhesion of *E. coli* ATCC 10536 and *E. coli* PCA. Additionally, the use of Formulation led to a reduction in the biomass biofilm in formation up to 80% and in biofilm mature reductions of 89.9% and 74.6% were observed for *E. coli* PNT and *E. coli* PCA, respectively. A decrease in CFU/mL was also observed in both formation and mature biofilms, with reductions of up to 91%. The Live/Dead microscopical images showed an evident disaggregation of the mature biofilm after treatment with Formulation. **Discussion and Conclusions:** This blend of active compounds could represent a promising and versatile approach to use in the treatment of UTIs due to *E. coli* to overcome the limitations associated with conventional therapies, because it could represent an efficacious and cost-effective strategy.

248 - The effect of resveratrol-based compounds on helicobacter pylori

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The effect of resveratrol-based compounds on *helicobacter pylori*

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Introduction: Resveratrol (RSV) is well known for its antimicrobial, anti-inflammatory and anti carcinogenic activities but with low bioavailability and solubility. The antimicrobial and antibiofilm activity of new synthetized RSV derivatives against reference and clinical *Helicobacter pylori* strains were demonstrated. The aim was to explore and detect new properties and mechanisms of action of new RSV derivatives regarding *H. pylori* gene expression, cellular membrane permeability and fluidity and the ability to penetrate mucin. Materials and Methods: The most acting derivatives named RSV3, RSV4 were studied against *H. pylori* strains to observe the following markers: i) cellular membrane permeability by Propidium iodide (PI) method; ii) membrane fluidity calculating Laurdan generalized polarization (GPexc) values and iii) gene expression of *motA*/*ureA* genes. Finally, an *in vitro* simulation model was performed to evaluate RSV3, RSV4 capability to penetrate mucin layer of gastric mucosa. Results: RSV4 changed the cellular permeability and fluidity of *H. pylori* and reduced *motA*/*ureA* gene expression. Permeability through mucin demonstrated that both derivatives can pass through different thickness mucin layers within 3 hours (RSV3 91% to 112%) and (RSV4 41% to 86%). Discussion and Conclusions: RSV3 and RSV4 were capable of affecting gene expression and membrane permeability/fluidity with high penetration through mucin layers, suggesting a promising antibacterial effect and increased bioavailability in the gastric mucosa.

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263 - Wastewater surveillance of foodborne and community-spread viruses in the city of Rome: a proof-of-concept study

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Wastewater surveillance of foodborne and community-spread viruses in the city of Rome: a proof-of-concept study

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Introduction Wastewater-based epidemiology is a powerful monitoring strategy to assess the circulation of viral pathogens at the community level. To date, however, few studies have provided an in-depth analysis of trends of circulation of multiple viral pathogens using this approach. The FoodZoo project is a proof-of-concept study that aims at assessing the use of wastewater monitoring to describe the epidemiology of foodborne and community-spread viruses in the population of Rome, Italy.
Materials and Methods Twenty-one samples were collected between October 2024 and January 2025 from three wastewater treatment plants in Rome, which collected waters from approximately 2 million inhabitants. Sample concentration was performed using a polyethylene glycol (PEG)/NaCl precipitation protocol and nucleic acids were extracted with the MagPurix automated platform. A panel of enteric viruses, namely norovirus genogroups I and II (NoVGI, NoVGII), adenovirus (AdV), astrovirus (AstV), enterovirus (EV), rotavirus (RV), Aichi virus (AiV), hepatitis A and E viruses (HAV, HEV), were tested for by digital (RT)PCR (QIAcuity, Qiagen) using published assays. Viral genome copies per L of wastewater (g.c./L) were normalized by the daily flow of each WTP and the viral load per inhabitant per day (g.c./day*inhabitant) was calculated.
Results The detection frequencies of the nine viruses in the study period varied from 100% (NoVGII, AdV, AstV, AiV, RV) to 81% (EV), 67% (NoVGI), 57% (HEV), 29% (HAV). Virus concentration (representing the intensity of virus circulation in the population) displayed high

variability, ranging from below the analytical limit of detection to 6.7 $\log_{10}$ g.c./day*inhabitant in the case of HAV, and from 10.9 to 11.4 $\log_{10}$ g.c./day*inhabitant for AdV. An increased circulation of AdV was observed between mid-November and mid-December, while NoVGII showed a steadily increasing trend from October through the end of January.

Discussion and Conclusions The preliminary results of this proof-of-concept study on wastewater surveillance of foodborne and community-spread viruses show significant differences in virus occurrence in the human population of each catchment area. Viruses as AdV, NoVGII, RV, etc showed sustained circulation during the whole study period, while other viruses occurred more sporadically and at lower concentrations, likely reflecting a lower number of individuals shedding the virus. These results suggest that the epidemiology of a given virus in a population may be modelled using data from wastewater monitoring. Further studies providing larger datasets and integrating trend analysis and molecular characterization of the detected viruses are needed to fully assess the feasibility of epidemiological surveillance through wastewater analysis.

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264 - Wastewater surveillance of bacterial foodborne diseases: tracking Salmonella, Listeria and STEC in Rome's population

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Wastewater surveillance of bacterial foodborne diseases: tracking *Salmonella*, *Listeria* and STEC in Rome's population

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Introduction Wastewater monitoring is increasingly recognized as a valuable tool to assess the circulation of human pathogens at the community level. While its use for viral diseases has been significantly exploited during the COVID-19 pandemic, its application for bacterial foodborne pathogens surveillance has been less explored. Herein we report the preliminary results of the FoodZoo project, a proof-of-concept study that aims at assessing the potential for wastewater monitoring to describe the epidemiology of zoonotic foodborne pathogens in the population of Rome, Italy.
Materials and Methods Thirty-one wastewater samples were collected between October 2024 and March 2025 from three wastewater treatment plants (WTPs) in Rome, representative of approximately 2 million inhabitants. Samples were shipped refrigerated to the laboratory and were tested for *Salmonella* spp., *Listeria* spp and *L. monocytogenes*, STEC (*stx1*, *stx2*) within 6 h of collection. Bacterial counts were performed using an MPN-PCR approach with preferential or selective media for pre-enrichment and Real-Time PCR for confirmation of positive tubes. Bacterial counts per liter of wastewater (cfu/L) were normalized by the daily flow of each WTP and the number of each pathogen per inhabitant per day (cfu/day*inhabitant) was calculated.
Results *Salmonella* spp. was detected in 30/31 (96.8%) of samples, followed by *Listeria* spp. (81.0%) and *L. monocytogenes* (52.4%). *Stx* genes of STEC were also frequently detected (71.0% of samples positive for *stx*1, 96.8% for *stx*2). The average concentration of the targets

was 6.5 $\log_{10}$ c.f.u./day*inhabitant for *Salmonella*, 5.6 and 5.2 for *Listeria* spp. and *L monocytogenes*, respectively, and 6.5 and 7.5 for *stx*1+ for *stx*2+ STEC, respectively. Significant spikes in pathogens concentrations were detected in some samples/WTPs occasionally (e.g. *stx*2+ STEC in mid-November), which might reflect a localized increase in pathogen shedding. Isolates of *Salmonella*, *Listeria* and STEC were taken from samples for further characterization and comparison with human, food and veterinary isolates.

Discussion and Conclusions The preliminary results of this study on foodborne pathogens in the urban wastewater of Rome confirm the different epidemiology of *Salmonella* and *Listeria*, with a significantly more frequent occurrence on the first one in the population. However, the results also highlighted a significant occurrence of *L. monocytogenes* and, even more substantial, *stx*1/*stx*2 STEC in wastewater, pointing, possibly, to an undetected (asymptomatic or paucisymptomatic) circulation in the population. Further studies are required to evaluate the feasibility of assessing the incidence of these zoonotic diseases in the human population using wastewater analysis.

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273 - Unmasking Mycobacterium bovis: new insights on Zoonotic Tuberculosis in Italy

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Unmasking *Mycobacterium bovis:* new insights on Zoonotic Tuberculosis in Italy

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Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a zoonotic disease within the Mycobacterium tuberculosis complex (MTBC) with major public health, veterinary, and environmental implications due to its cross-species transmission and complex ecology. It remains endemic in many low- and middle-income countries and poses challenges even in high-income regions like parts of Europe. Accurate diagnosis is crucial for patient management and effective public health responses. Misidentifying *M. bovis* among MTBC members can lead to inappropriate treatment, as *M. bovis* is resistant to Pyrazinamide, a first-line anti-tuberculosis drug. This study aimed to characterize *M. bovis* isolated from patients with varying clinical presentations.

Materials and methods (761)

We analyzed Mycobacterial isolates from MGIT broth, obtained from tuberculosis-suspected patients enrolled between 2020–2024 in hospitals across Forlì-Cesena, Rimini, and Ravenna. *M. bovis* presence was detected using GenoType® MTBC, with DNA confirmation via real-time PCR targeting RD4. Isolates were genotyped by spoligotyping and MIRU-VNTR. Libraries were prepared using Illumina DNA Prep, (M) Tagmentation Kit and sequencing was performed on Minister system (Illumina, San Diego, United States). Raw reads were checked with FastQC

(v.0.12.1) and filtered with Trimmomatic (v.0.39). Filtered reads were analyzed with TBprofiler (v.4.4.2) and RD scan (v.1.0.5). MTBseq pipeline (v.1.0.3) in combination with iqtree was used to perform phylogenetic analysis.

Results

From 2020 to 2024 six *M. bovis* strains (BO1-6). were isolated from different pathological samples All strains shared Pyrazinamide resistance. BO1 (La1.2 strain) showed 453338453365SB0120 profile, unique in the whole ITAN-TB database, with Streptomycin resistance. BO2 (La1.2 strain) has a new spoligotype pattern and 4553310344385 profile: this profile, with different spoligotype, was previously found in bovine samples from Sicily. BO3 resulted as *M.bovis* La1.2 BCG strain with 5551310354355SB0120 profile, shared with human and vaccine strains. BO4 (La1.7.1 strain) had 5453310254385SB0121 profile: with different spoligotype previously found in human samples; with Ethionamide resistance. BO5 and BO6 had incomplete genotypes, respectively 555NA310444365SB0120 and NA35NA310254365SB0125. All strain identifications were confirmed by phylogenetic analysis.

Discussion and conclusion

Genomic characterization revealed that La1.2 strains, mainly circulating in Italy, were isolated from mediastinal lymph nodes, while La1.7.1, widespread in North Africa, was found in a patient with latero-cervical lymphadenitis. *M. bovis* BCG strain from mesenteric lymph nodes excluded iatrogenic origin. Findings highlight the need for zoonotic TB surveillance and One Health collaboration for effective identification and management.

278 - Virulence traits, antimicrobial resistance, and clonal relatedness of Staphylococcus aureus strains isolated from subclinical mastitic milk from dairy ruminants in Sicily

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Virulence traits, antimicrobial resistance, and clonal relatedness of *Staphylococcus aureus* strains isolated from subclinical mastitic milk from dairy ruminants in Sicily

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Introduction

Staphylococcus aureus is the most clinically significant species within the *Staphylococcus* genus, responsible for various diseases in humans and animals, for which antibiotic therapy may be ineffective. Given its ability to be commensal, colonizing, latent, or disease-causing, and its aptitude to switch between host species and from colonization to invasion, *S. aureus* poses a significant challenge as a food-borne bacterium. For dairy ruminants, it is one of the most common causes of mastitis worldwide, both clinically and subclinically.

Materials and Methods

We analyzed 101 *S. aureus* isolates obtained from the quarter milk of animals affected by subclinical mastitis in the Ragusa area (Sicily, Italy). We tested phenotypic resistance against nine antibiotics using the Kirby-Bauer method, while determining the Minimum Inhibitory Concentration (MIC) values for oxacillin (OXA) and vancomycin (VA). Additionally, we genetically characterized the isolates through PCR multiplex (for the presence of *spa, mec*A*, mec*C*, pvl, van*A*, van*B*,* and *van*C genes), Pulsed-Field Gel Electrophoresis analysis, and multi-locus sequence typing (MLST).

Results

Overall, we observed the highest rates of antibiotic resistance against gentamicin (47.5%) and erythromycin (29.7%), with 86.1% of strains showing resistance to at least two antimicrobials and 33.7% exhibiting resistance to three antimicrobial classes. No correlations were found between the occurrence of antibiotic resistance genes, phenotypic resistance, and phylogenetic analysis, revealing different phenotypic resistance profiles even within the same PFGE cluster. Lastly, in addition to the newly detected ST947 sequence type, MLST analysis identified five other STs, clustering them into three CCs, with CC5, which originates from human ancestral strains that have undergone human-to-animal host jumps, being the dominant group.

Discussion and Conclusions

In the present study, the high incidence of resistance against critically important antimicrobial agents raises concerns about the antibiotic resistance of *S. aureus* species, demonstrating that raw milk may be a source of both MDR and virulent strains, and that some lineages may result from human-to-animal host-switching events. Furthermore, the observed clonality and resistance pattern diversity, even within the same pathogenic lineage, confirms the species' high genomic plasticity and, in turn, the significant probability of horizontal gene transfer. The convergent evolution, evidenced by both the low number of CCs and the newly discovered ST, further underscores the species' great ability to adapt to environmental challenges, making the dairy farm a potential reservoir of pathogenic bacteria that can rapidly switch between animals and humans, and vice versa. Our study provided valuable insights into regional trends, allowing for the identification of significant patterns and offering an understanding of bacterial dynamics in these environments.

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280 - Conserved FAD/NAD(P)H-Dependent Dehydrogenases in Pathogens and Hosts: A Structural Framework for Selective Antimicrobial Design

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Conserved FAD/NAD(P)H-Dependent Dehydrogenases in Pathogens and Hosts: A Structural Framework for Selective Antimicrobial Design

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FAD/NAD(P)H-dependent dehydrogenases are essential for the redox metabolism of bacteria, fungi, and protists, supporting key processes such as respiration, detoxification, and survival under host-imposed stress. Despite notable sequence diversity, these enzymes exhibit overall structural similarity, with high conservation of residues in their catalytic cores, across a wide range of pathogenic microbes and their mammalian hosts, posing a major challenge for selective antimicrobial drug development (1). This structural resemblance presents a critical microbiological challenge: while these flavoproteins are indispensable for pathogen survival and thus represent promising antimicrobial targets, their high similarity to human homologs complicates drug development by increasing the risk of off-target effects and host toxicity. In this study, we perform a comparative structural analysis of FAD/NAD(P)H-dependent dehydrogenases across pathogens and mammals, focusing on enzymes such as type II NADH dehydrogenases and dienoyl-CoA reductase (1,2), which are primarily found in pathogenic microorganisms, versus apoptosis-inducing factor (AIF) (1), which appears to be specific to mammals. We also examine structurally conserved enzymes like thioredoxin reductase, glutathione disulfide reductase, and dihydrolipoamide dehydrogenase, which are shared across kingdoms (1,3), to identify structural features that could be exploited for selective antimicrobial targeting. In our analyses we underscore the need to develop highly selective inhibitors capable of disrupting microbial redox metabolism without interfering with homologous human enzymes. A detailed understanding of the structural and functional features among FAD/NAD(P)H-dependent dehydrogenases can be essential for the rational design of next-generation antimicrobials. Such insights will be pivotal for achieving therapeutic selectivity, minimizing host toxicity, and addressing the growing challenge of infectious diseases and antimicrobial resistance.

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Patent Application N. 102019000022545 Title: ENG “Method for the identification of FAD/NADH dependent dehydrogenase binding regions in mammalia and mammalia/plant pathogens, to be employed for drug-design.

286 - Hermetia illucens-Derived Chitosan: A Promising Immunomodulatory Agent for Applications in Biomedical Fields.

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***Hermetia illucens*-Derived Chitosan: A Promising Immunomodulatory Agent for Applications in Biomedical Fields.**

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Introduction: Chitin and chitosan are two biopolymers of great interest, thanks to their versatility and potential use in various application fields. However, processing chitin is complicated because it is not soluble in water and in common organic solvents. For this reason, chitin is typically converted into its more soluble derivative, chitosan, through a deacetylation process that can be either homogeneous or heterogeneous. Currently, the

demand for the biopolymer on the market is growing, and, for this reason, it is important to biologically characterize the biopolymer produced from an alternative source to crustaceans In this perspective, insects, thanks to their abundance and biodiversity, represent a valid solution to this problem. Among this, the bioconverter *Hermetia illucens* is of great interest. This fly feeds on agri-food byproducts, converting them into larval biomass, rich in numerous molecules of high biological value, including chitin. Moreover, the farms produce a vast amount of waste products, like pupal exuviae, released from molting processes and dead adults, which are rich in chitin. They are thus recovered within a fully circular, zero-waste economy process. In this way, the extraction of chitin and the production of chitosan become entirely and highly sustainable procedures. ***Material and Methods:*** HaCat cells were treated with *Salmonella enterica* subsp. *enterica* serovar Typhimurium LPS for 6 and 24 hours with or without chitosan obtained by both heterogeneous and homogeneous deacetylation from larvae, pupal exuviae, and adults insects. Real-Time PCR and ELISA essay were performed to evaluate the ability of the biopolymer to downregulate the expression of proinflammatory cytokines IL-6, IL-8, IL-1 alpha and TNF-alpha. ***Results***: insect chitosan samples effectively downregulated cytokine expression, with improved activity obtained from heterogeneous chitosan treatments. ***Discussion and Conclusions:*** this work highlighted the potential of *H. illucens* as a source of chitin and chitosan, for applications in the biomedical and pharmaceutical fields.

288 - Pseudomonas aeruginosa detection, antimicrobial susceptibility evaluation and genomic characterization in hospitals and workplaces water samples of Campania Region

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Pseudomonas aeruginosa detection, antimicrobial susceptibility evaluation and genomic characterization in hospitals and workplaces water samples of Campania Region

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Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that can form biofilms and, consequently, be detected in water system pipelines. It is among the pathogens most responsible for nosocomial infections and causing pneumonia. Multidrug-resistant and extensively drug-resistant (MDR/XDR) *P. aeruginosa* has become increasingly widespread in recent years, constituting a public health concern. For this reason, the aim of this study was to monitor bacterium presence in water samples of Campania region, evaluate antimicrobial susceptibility and provide genomic characterization.

Materials and Methods

393 total drinking water samples were obtained from taps in 21 hospitals (286 samples of them) and 26 workplaces (107 samples of them) in Campania region, in the period March - September 2024. Sampling was conducted according to UNI EN ISO 19458:2006 and microbiological analysis according to UNI EN ISO 16266:2008. Suspected colonies of *P. aeruginosa* were confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), antimicrobial susceptibility (against aminoglycosides, penicillins,

monobactams, cephalosporins, carbapenems, fluoroquinolones, miscellaneous agents, tetracyclines) was tested by broth microdilution method and genomic characterization was performed by whole genome sequencing (WGS). Multi-locus sequence typing (MLST) for sequence types (STs) investigation was conducted through the PubMLST database.

Results

A total of 47 out of 393 samples (12.0%) were positive for *P. aeruginosa*, of which 26 (9.1% out of 286 samples) from hospitals and 21 (19.6%, of 107 samples) from workplaces. Cefazolin, cephalotin (1st generation cephalosporin), cefoxitin, cefuroxime (2nd generation), cefpodoxime (3rd generation), ampicillin and ampicillin/sulbactam resistance was observed in all positive samples (100%) and cefepime (4th generation) resistance in 6 samples (12.8%). MDR was observed in 16 samples (34.0%). The analysis revealed the presence of 13 different STs, of which ST 309 was the most represented. In addition, this study revealed the presence of the same clone in different wards of the same hospital and of the same clone in hospital and non-hospital settings.

Discussion and Conclusions

This study highlights important findings of environmental monitoring. Firstly, the same clone detected in different places reveals its diffusion in different pipelines. Secondly, it is important to monitor microbial presence in the environment, in particular in facilities hosting vulnerable people. Continuing in this direction could prevent human infections and safeguard public health.

324 - Tracking Antibiotic Resistance Genes in Foodborne Bacteria: A One Health Approach

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Tracking Antibiotic Resistance Genes in Foodborne Bacteria: A One Health Approach

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1. Introduction

Antibiotic resistance is a major public health concern that extends beyond clinical settings to include animals, food, and the environment. Monitoring antibiotic resistance genes (ARGs) in foodborne bacteria is crucial for identifying and tracking these resistant strains, thus enabling the development of effective health policies aimed at preventing their spread within the One Health framework.

2. Materials and Methods

We assessed the presence of thirty-one ARGs in 76 strains isolated from food samples exhibiting intermediate or resistance to at least one antibiotic, as determined by the Kirby-Bauer disk diffusion test. Molecular analyses were performed to evaluate the presence of ARGs conferring resistance to beta-lactam antibiotics (*blaCTX-M IV, blaTEM, blaOXA, blaSHV, blaCMY II, blaCTX M I, blaCTX M II, blaDHA*), tetracyclines (*tetA, tetB, tetC, tetD, tetE, tetG, tetO, tetW*), quinolones (*qnrA, qnrB, qnrC, qnrD, qnrS*), sulphonamides (*sul-I, sul-II, sul-III*), carbapenem *(vim1, vim2, ndm)* and cloramphenicol (*cat1, cat2, floR, dfloR*).

3. Results

The most common beta-lactams resistance gene was *blaTEM* , detected in 14 out of 76 samples (18%). Among 21 quinolone-resistant strains, *qnrD* was present in 2 isolates (10%). In the 22 tetracycline-resistant strains, *tetA* was found in 7 (32%), and *tetW* in 4 (18%), while *tetB, tetC, and tetD* were each found in -one single strain. Out of the 11 sulfonamide-resistant strains, 36% carried the *sulI* gene and 18% had the *sulII* gene. A positive correlation was seen between the *qnrB and sulII genes* ($R = 0.70$, $P<0.001$).

4. Discussion and Conclusions

The identification of multilple resistance genes in foodborne bacteria highlights various antibiotic resistance mechanisms in non-clinical environments. The *blaTEM* gene was the most frequently detected beta-lactamase gene indicating its widespread distribution. A significant correlation ($R = 0.75$, $P < 0.001$) was observed between the *tetB* gene and both *qnrS* and *qnrD* suggesting potential colocalization on mobile genetic elements, such as plasmids or trasposons. These findings reinforce the need for continuous molecular surveillance to track the spread of resistance and support evidence-based interventions within the One Health approach.

329 - GUT MICROBIOTA PROFILE IN PHARMACOLOGICALLY TREATED SUBJECTS WITH BIPOLAR DISORDER

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Abstract – SIM 2025

GUT MICROBIOTA PROFILE IN PHARMACOLOGICALLY TREATED SUBJECTS WITH BIPOLAR DISORDER

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Introduction: The pharmacogenomics of Lithium (Li), the mainstay therapy in Bipolar Disorder (BD), has been extensively studied, but so far none of the suggested molecular targets have

been supported as pharmacological or clinically relevant markers. Increasing evidence suggests that the gut microbiota may be involved in modulating the response to psychotropic medications. Our aim was to identify microbial biomarkers associated with Li response in BD. To this end, the intestinal microbial profile of patients treated with Li (n.= 40) was compared with that of a control group (n.= 37) treated with another mood stabilizer (valproic acid).

Materials and Methods: Each patient collected a fecal sample. Genomic libraries were constructed by clonal amplification of the V3-V4 hypervariable region of the gene encoding the 16S rRNA, which were sequenced using the Illumina MiSeq platform.

Results: The alpha diversity analysis showed that the Evenness index was statistically significant (q=0.005), highlighting a significantly less uniform distribution of microbial abundance in patients treated with Li compared to those treated with valproic acid. As for beta diversity, only the weighted UniFrac (q=0.037) and unweighted UniFrac (0.038) dissimilarity indices were statistically significant, which highlight a qualitative and quantitative diversity of the different bacterial taxa between the two study groups. Analysis of differential abundance-ANCOM-BC following FDR and covariates correction showed the significant increase and reduction of different bacterial taxa in the comparison between the two study groups.

Discussion and Conclusions: The microbial community of patients treated with Li is significantly different from that of patients treated with another blood stabilizer. Further studies are currently underway to investigate whether the effect of Li treatment is limited to patients who do not respond to pharmacological treatment.

337 - Faecal microbiota transplantation from WAG/Rij rats chronically treated with sodium butyrate alleviates epileptic phenotype in recipient rats

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Faecal microbiota transplantation from WAG/Rij rats chronically treated with sodium butyrate alleviates epileptic phenotype in recipient rats

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Introduction: Epilepsy is a prevalent neurological disorder, with approximately 30% of patients remaining unresponsive to anti-seizure medications, highlighting the need for alternative therapeutic strategies. Faecal Microbiota Transplantation (FMT) is being investigated as a potential treatment for epilepsy. Clinical studies are exploring if FMT can modulate behaviour and improve cognitive function in epilepsy patients, including those with drug-resistant epilepsy. The postbiotic sodium butyrate, a short-chain fatty acid produced by the microbial fermentation of dietary fibres in the gut, has garnered significant interest for its potential therapeutic effects also in epilepsy. This compound not only serves as a crucial energy source for colonic epithelial cells but also exhibits anti-inflammatory and neuroprotective effects through gut microbiota modulation. In a previous study, we demonstrated that FMT from healthy Wistar rats significantly decreased the number and duration of seizures in recipient WAG/Rij rats. Here we investigate, whin the same genotype, the effects of FMT from WAG/Rij rats chronically treated with sodium butyrate to alleviate absence epilepsy into untreated WAG/Rij recipients rats.
Materials and methods: Sodium butyrate (30 mg/kg/day) was administered to the rats via drinking water for 6 months, starting prior to seizure onset. Electroencephalography (EEG) recordings were performed at various time points, and faecal samples were collected for metataxonomic analysis. FMT was conducted 3 times per week for 4 weeks, after which EEG recordings and GM composition were analysed in the recipient rats.
Results: Results showed that gut microbiota remodelling in donors WAG/Rij rats due to butyrate treatment strongly impacted recipients rats after FMT, decreasing seizure severity. Microbiota analysis revealed distinct compositional changes, with higher abundance of *Akkermansia*, *Bacteroides* and *Prevotellaceae NK3B31 group* in FMT recipients, and increased

Muribaculaceae and *Eubacterium coprostanoligenes group* in vehicle-treated controls. Network analysis indicated a clear shift in microbial community structure, with *Akkermansia* and *Bacteroides* negatively associated with taxa enriched in controls. These genera were also negatively correlated with seizures metrics. This intra-strain FMT significantly reduced seizure activity, supporting the idea that stabilisation of gut ecosystem alone is sufficient to confer neurological effects, even in the absence of further drug treatment.
Conclusions: These findings point toward the potential of functional “autologous” FMT based on microbiota reshaping by targeted treatments in order to limit side effects and tolerance to therapies.

343 - Integrating Wastewater Surveillance and Machine Learning for Predicting COVID-19 Cases: A One Health Strategy

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Integrating Wastewater Surveillance and Machine Learning for Predicting COVID-19 Cases: A One Health Strategy

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Introduction and Aim

Wastewater-based epidemiology (WBE) offers a unique and inclusive approach to monitor infectious diseases at the community level. As a One Health tool, WBE integrates environmental and epidemiological factors, emphasising the necessity for a multidisciplinary approach. Unlike clinical surveillance, which may underrepresent asymptomatic and unreported cases, WBE captures viral shedding from the entire population, providing a comprehensive overview of infection dynamics.

This study explores the potential of combining WBE with bioinformatics as a predictive tool for COVID-19 cases, underscoring its interdisciplinary value in infectious diseases surveillance.

Materials and Methods

From February 2023 to February 2024, a total of 53 wastewater samples were collected weekly at the wastewater treatment plant (WWTP) of Sondrio (Lombardy Region, Italy) and concentrated using polyethylene glycol precipitation. SARS-CoV-2 RNA was quantified using Real-Time qPCR, targeting the N1 and N3 gene regions. Results were expressed as average viral load between the N1 and N3 gene targets. Weekly COVID-19 case data were retrieved from the regional repository collecting information on SARS-CoV-2 nasal swabs limited to people living in the catchment area of the WWTP of Sondrio and expressed as incidence of positive cases. A machine learning model, XGBoost, was applied to predict the incidence of COVID-19 cases based on the temporal trends of viral load in wastewater.

Results

A strong correlation was observed between the load of SARS-CoV-2 RNA in wastewater and the incidence of COVID-19 cases. The XGBoost model showed high predictive accuracy (Figure 1), underscoring the utility of WBE in predicting infectious diseases. Moreover, the model's self-training suggests that incorporating additional data could further enhance its predictive accuracy.

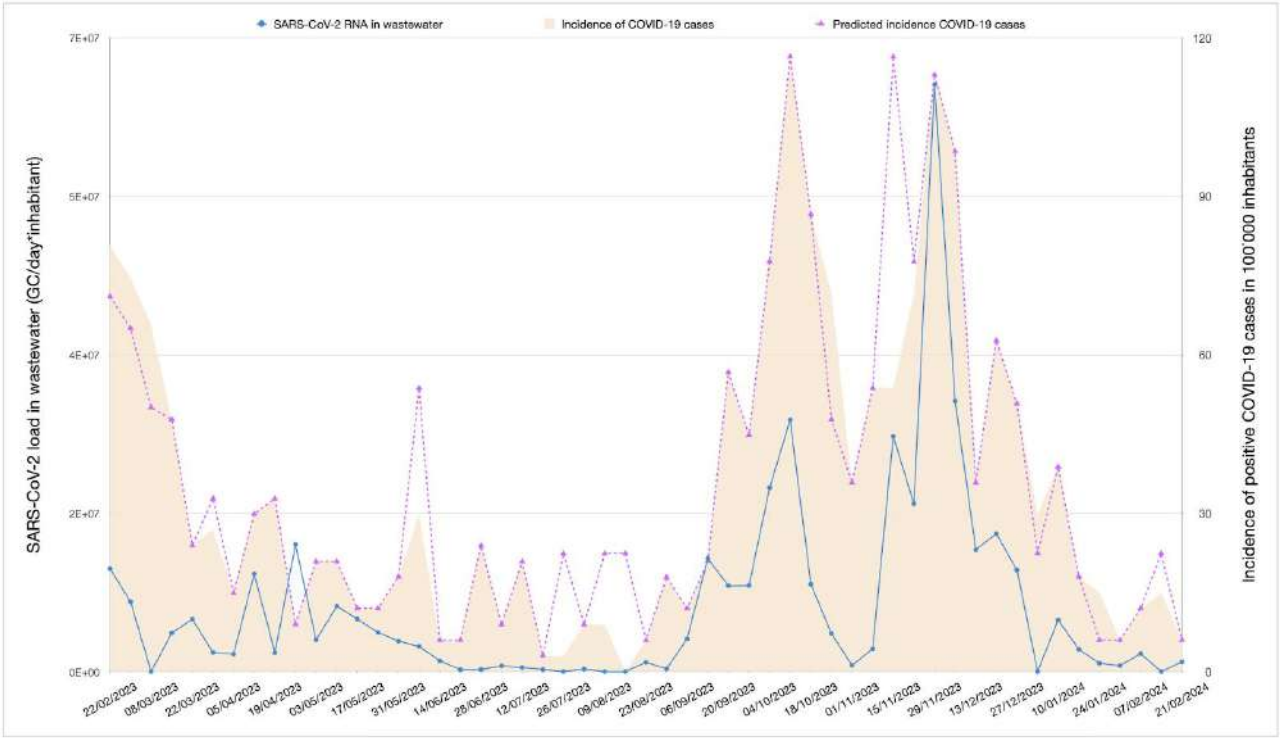

Figure 1. Weekly trends in the quantification of SARS-CoV-2 RNA in wastewater, incidence of COVID-19 cases reported by clinical surveillance and incidence of COVID-19 cases predicted by the model.

Discussion and Conclusions

This study highlights the relevance of wastewater surveillance as a multidisciplinary approach to infectious disease monitoring. By integrating environmental, public health, and data science perspectives, WBE can serve as a tool for tracking infectious outbreaks. Although the current

model is tailored to SARS-CoV-2, ongoing research is focused on developing a multivirus predictive framework capable of monitoring and forecasting multiple respiratory pathogens.

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