SIM
SOCIETÀ ITALIANA
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UNIVERSITÀ
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DELLA SOCIETÀ ITALIANA DI MICROBIOLOGIA SIM

8-11 SETTEMBRE 2024

PAVIA | UNIVERSITÀ DEGLI STUDI DI PAVIA

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PAVIA

ABSTRACT BOOK

SOMMARIO

T01 APPROCCI DIAGNOSTICI

3 - THE USE OF BACTERIAL VOLATILE ORGANIC COMPOUNDS DETECTION IN GRAM-NEGATIVES ANTIMICROBIAL SUSCEPTIBILITY TESTING

Maddalena Calvo *(1)* ***- Gaetano Maugeri*** *(1)* ***- Giuseppe Migliorisi*** *(2)* ***- Guido Scalia*** *(1)* ***- Stefania Stefani*** *(3)*

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The use of bacterial volatile organic compounds detection in Gram-negatives antimicrobial susceptibility testing

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Introduction. Volatile organic compounds (VOC) are small metabolites produced during bacterial growth. Although some challenges, their detection in microorganisms identification has been extensively studied (electric nose and ion-mobility spectrometry). Otherwise, ultimate technologies propose VOC colorimetric arrays in antimicrobial susceptibility testing (AST) due to the ability to discriminate between susceptible and resistant microbial isolates. On that premise, we evaluated the Vitek Reveal System (Biomerieux, Florence, Italy) directly on Gram-negative blood cultures during a prospective study.

Materials and methods. We enrolled 40 blood samples from patients recovered in the Policlinico of Catania (October 2023-January 2024). After a positive flag and the microscopic Gram-negative observation, the samples underwent a rapid molecular or spectrometric identification (ID), followed by rapid phenotypic antimicrobial susceptibility testing (AST) through the Vitek Reveal. The system included a 7-sensor array able to react with the organic volatile compounds on the headspace of each inoculated well. The analyzed samples were simultaneously processed through culture exams for standard turbidimetric AST. Graph 1 summarizes the overall applied procedures. The MIC results interpretation followed the EUCAST guidelines.

Results. The workflow mainly screened Klebsiella pneumoniae (18), Escherichia coli (11), and Acinetobacter baumannii (8) susceptibility profiles, including high percentages of carbapenem-resistant isolates. Otherwise, Enterobacter cloacae, Proteus mirabilis and Pseudomonas aeruginosa were rarely isolated. The Reveal essential agreement (EA) to the conventional AST was 100%, while the categorical agreement (CA) resulted in 99%. Specifically, the analysis revealed two very major errors (VME) for meropenem/vaborbactam in K. pneumoniae and amoxicillin/clavulanic

acid in E. coli. Graphs 2 and 3 illustrate a time to report (TTR) and turn-around time (TAT) analysis referred to our samples.

Discussion and conclusions. The Vitek Reveal system represents a novelty in the field of AST, showing high accuracy and categorical agreement with the standard of care. Furthermore, the technology was challenged by muti-drug-resistant microorganisms, demonstrating high sensitivity in defining MIC values. Although further studies will be essential to investigate less common infection aetiological agents, the system allowed a significant TAT reduction. In conclusion, susceptibility testing based on VOC are a fundamental challenge within our high-risk epidemiological area, where carbapenem-resistant Gram-negatives hold a warning primate.

16 - MOLECULAR SCREENING OF MYCOBACTERIAL INFECTIONS IN A LARGE ACADEMIC HOSPITAL IN ROME

Fabiana Diaco *(1) - Chiara Nonne (1) - Luisa Torrini (1) - Daniele Emanuele Compagnino (1) - Federica Sacco (2) - Agnese Viscido (2) - Ilaria Torrente (2) - Guido Antonelli (1) - Giammarco Raponi (3)*

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Molecular screening of mycobacterial infections in a large Academic Hospital in Rome

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Introduction

Routine diagnosis of infections sustained by alcohol-acid resistant microorganisms is challenging in clinical microbiology laboratories. The classic approach comprises the execution of bacterioscopy and cultures implying delayed diagnosis. Therefore, newer procedures and biomolecular methods have been introduced in the routine diagnosis. In our laboratory, we are experiencing an approach based on the determination of genome coding for tuberculous (MTB) and non-tuberculous (NTM) Mycobacteria directly on clinical respiratory samples.

Materials and Methods

In the Clinical Laboratory for Microbiology of the Academic Hospital Policlinico Umberto I in Rome, 153 respiratory samples were analysed in the period February-April 2024. The samples (63 broncho-alveolar lavages, 54 sputum, 19 pleural fluids, 17 tracheo-bronchial aspirates) were firstly decontaminated with M10 sputum pretreatment Kit (SD Biosensor, Republic of Korea) and thereafter processed with the SD BIOSENSOR MTB/NTM panel in the STANDARD M10 instrument (SD Biosensor). The MTB/NTM panel reveals the sequences IS6110 for MTB and ITS region for NTM. The positive samples for the IS6110 sequence were further processed with the SD BIOSENSOR MDR-TB panel in order to reveal genes coding for the resistance to rifampicin (rpoB1, rpoB2, rpoB3, rpoB4) and isoniazid (katG, inhA). All the samples were also cultured in Lowenstein-Jensen (Becton Dickinson Italia S.p.A.) and NTM Elite agar (Biomerieux Italia s.p.a.) for 40 and 15 days respectively at 37°C. Colonies grown on NTM agar were identified by MALDI-TOF MS (Bruker Daltonik). MTB colonies grown on Lowenstein-Jensen media were observed by Ziehl-Neelsen staining and confirmed by Xpert® MTB/RIF Ultra (Cepheid, Italia).

Results

Of 153 samples analysed 5.98% tested positive. Specifically, the sequence IS6110 for MTB was detected in 5.23%, ITS region for NTM in 0.65% and 2.61% of the samples was invalid (Figure 1). The positive samples belonged to 66.67% of male and to 33.33% of female, with an average age respectively of 55 and 36 years. Only in one MTB positive sample were detected the mutation of katG

and inhA genes. Finally, the culture test confirmed the results in 50% of MTB/NTM of positive samples, 40% are still ongoing and 10% showed negative results (Figure 2).

Discussions and conclusions

Our diagnostic approach seems to be rapid, simple and accurate for the identification of mycobacterial infections and their possible genetic resistance to the most commonly used drugs in the clinical setting. Rapid diagnosis might allow the clinician in the prompt isolation of the patient and in the therapeutic treatment.

Figure 1. Results in percentage of samples analysed with SD BIOSENSOR MTB/NTM.

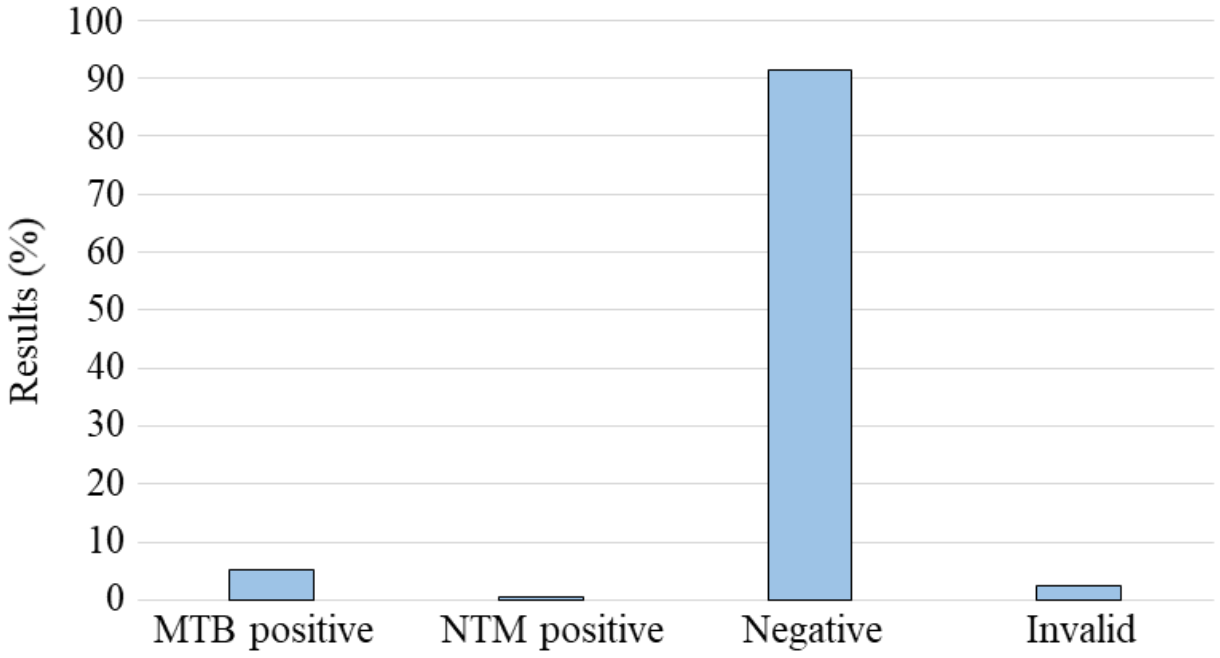
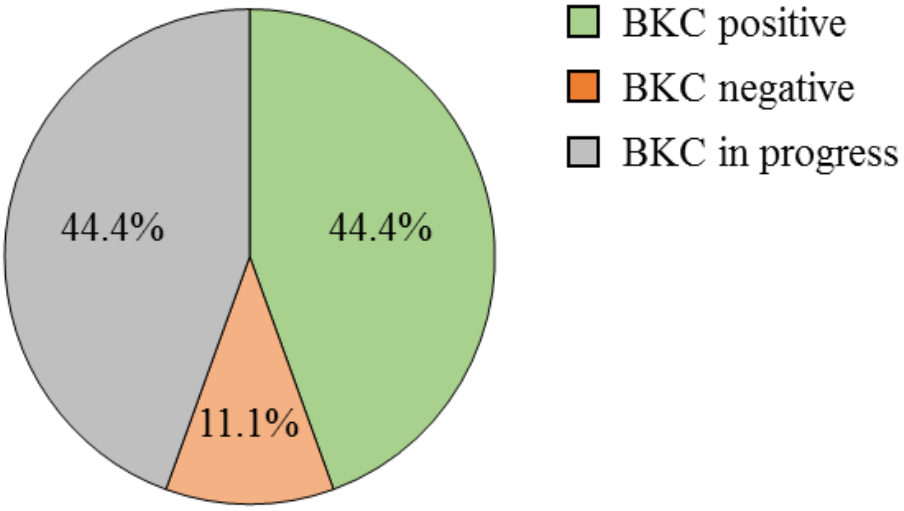


Figure 2. Percentage of positive samples confirmed by culture.


BKC positive
BKC negative
BKC in progress
44.4%
44.4%
11.1%

17 - MEROPENEM AND CEFTAZIDIME/AVIBACTAM SUSCEPTIBILITY AS INDICATORS OF CARBAPENEM-RESISTANCE: A REAL-LIFE DIAGNOSTIC EXPERIENCE INTEGRATING AN IMMUNO-CHROMATOGRAPHIC ASSAY.

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Meropenem and ceftazidime/avibactam susceptibility as indicators of carbapenem-resistance: a real-life diagnostic experience integrating an immuno-chromatographic assay.

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Introduction. Carbapenem-resistant Enterobacterales represent a dominant health problem, especially in endemic regions such as Southern Italy. The high prevalence rate of these strains inspired new diagnostic algorithms for carbapenemases identification. We evaluated the immuno-chromatographic KPC/IMP/NDM/VIM/OXA-48 Combo Test kit (Jengsu Medomics Medical Technology co., ltd) in carbapenemases detection, comparing its performance to regular molecular techniques.

Materials and methods. The study settled at the University Hospital Policlinico of Catania, where the samples were inoculated on culture media, with a ceftazidime and meropenem (MEM) disks screening on surveillance samples. All the MEM-resistant Enterobacterales from surveillance swabs and all the MEM-resistant or susceptible ones from all samples underwent identification (MALDI Biotyper® Sirius, Bruker Daltonics) and phenotypic antibiogram. All the MEM-resistant strains experienced the Combo test and the molecular assay (Cepheid GeneXpert). The MEM susceptible isolates took the same workflow in the case of a ceftazidime-avibactam (CAZ/AVI) resistance. The study enrolled some MEM-susceptible Enterobacterales as a negative control. All the CAZ/AVI resistant and MEM susceptible KPC strains went through next-generation sequencing (NGS, Illumina, San Diego, CA, USA), suspecting the presence of variants.

Results. The analysis reported 62 carbapenemases-producing strains, accounting for 60 Klebsiella pneumoniae, which revealed 50 KPC, 6 NDM, 2 NDM/KPC, 1 NDM/OXA- 48, and 1 VIM/OXA-48. Furthermore, 1 NDM/KPC Escherichia coli and 1 NDM Proteus mirabilis emerged. The Combo test and the Cepheid assay gathered the same results. After the NGS, the selected CAZ/AVI resistant and MEM susceptible KPC K. pneumoniae strains showed KPC-3 (16), KPC-31 (7), and KPC-34 (2), which were

identified by the Combo test. Otherwise, 54 MEM- susceptible Enterobacterales tested negative on both the techniques. The immunochromatographic results always matched the molecular investigations.

Discussion and conclusions. The immunochromatographic assay showed a significant agreement percentage to the molecular assay, which currently is the standard confirmation test for carbapenemases detection. Moreover, the test requires a minimum interval to gather a result. The test identified different KPC variants (KPC3, 34 and 31), which occasionally furnished false negative results (KPC-31) during previous studies. Our epidemiology revealed double carbapenemases, which are difficult to detect without a molecular assay. However, the Combo test identified all the double genes. In conclusion, we suggest its inclusion in the diagnostic workflow to promptly gather resistance data, allowing clinicians to plan infection control measures or apply targeted therapies.

19 - RAPID MRSA/MSSA SURVEILLANCE: A SIX-YEARS DUAL MOLECULAR-CULTURAL APPROACH

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Rapid MRSA/MSSA Surveillance: A Six-Years Dual Molecular-Cultural Approach

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Introduction

Lack of standardization and long turnaround-times of standard cultural-approaches may limit the effectiveness of active-surveillance strategies aimed at identifying methicillin-resistant and susceptible Staphylococcus aureus (MRSA/MSSA) nasal-carriers as to establish prompt clinical interventions in hospital settings. Here we describe a six-year diagnostic performance of a simultaneous molecular and cultural MRSA/MSSA-screening approach.

Material and Methods

From Jan-2017 to Dec-2022, 12,048 samples from 10,328 at-risk patients were preemptively screened at IRCCS Humanitas Research Hospital by RT-PCR (Xpert® SA Nasal Complete, Cepheid-US) together with cultural-assay (chromogenic-agar), at admittance in orthopedics and surgery wards, intensive-care units (ICUs), and upon clinical judgment in remaining wards. RT-PCR MRSA-positive patients were immediately cohorted. Phenotypic susceptibility testing was performed by automated microdilution-assay (PhoenixTM).

Results

MRSA-colonization rate was 1.7% in orthopedic/surgical-wards and 3.4% in ICUs. MSSA was detected in 22.3% and 25.2% of samples, respectively. At-risk patients screened in Emergency Department (ED) showed the highest MRSA and MSSA-colonization rates (13.2%-28.2% respectively; $p<0.001$ vs. orthopedic/surgical-wards). Clinical-wards had intermediate MRSA-rates (7.6%) and the lowest MSSA-rates (18.9%; $p<0.001$ vs. orthopedic/surgical-wards). MRSA/MSSA colonization trends were stable over the six years analyzed. RT-PCR and standard cultural-assay were 97.0% concordant (11,681/12,048), but RT-PCR provided results in a median of 2.9 hours vs. 50.1 hours for isolate-identification and susceptibility-testing ($p<0.001$). Discordances were for 91.6% RT-PCR over detections (N=336), predominantly of MSSA (85.7%, N=288). Per-sample sensitivities of RT-PCR for culture-proven colonization was 98.0% (95% CI:97.7-98.2) and 99.8% (95% CI:99.7-99.9), respectively. During hospitalization, 81/3142 (2.6%) colonized patients developed a S. aureus bacteremia, vs 25/8906 (0.3%) non-colonized ($p<0.001$). MRSA-colonized patients had a significantly higher incidence of MRSA BSIs then MSSA-colonized for MSSA BSIs (5.4% [22/406] vs. 1.9% [52/2732]; $P<0.01$). Incidence of MRSA/MSSA BSIs in the 8910 non-colonized patients was extremely low (0.1 and 0.2%, respectively).

Discussion and Conclusions

Molecular and cultural approaches for MRSA/MSSA screening were highly concordant, yet with a significant advantage for RT-PCR in terms of laboratory turn-around-time and, consequently, time to clinical interventions. Colonized patients had higher risk of S. aureus BSIs, highlighting the importance of improving the effectiveness of control and decolonization strategies, beyond classical risk categories.

20 - ACTIVE SURVEILLANCE OF PATIENTS COLONIZED WITH CARBAPENEM-RESISTANT ENTEROBACTERALES: RESULTS OF COMBINED MOLECULAR-CULTURE PROTOCOL AT FONDAZIONE IRCCS CA' GRANDA OSPEDALE MAGGIORE POLICLINICO, MILAN

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Active surveillance of patients colonized with carbapenem-resistant Enterobacterales (CRE): results of combined molecular-culture protocol at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan.

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Introduction

Carbapenem-resistant Enterobacterales (CRE) are among the bacteria that need urgent attention globally. Active surveillance programs upon admission are essential for the early identification of possible CRE carriers and the timely adoption of measures to contain the spread. The study aims to analyze the epidemiology of CRE identified by a molecular approach in rectal swabs of patients at hospitalization.

Materials and methods

From January 1st to April 19st 2024, 1,630 rectal swabs from patients admitted at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) were screened for CRE in the first 24 hours of

hospitalization by a molecular approach (MA, multiplex RT-PCR AllplexTM Entero-DR Assay, Seegene), able to detect the following resistance genes: KPC, NDM, OXA48, VIM, IMP. All rectal swabs tested positive or invalid with MA were subsequently analyzed with the conventional approach (CA), based on inoculation of 100 µL on Agar MacConkey, CHROMID® CARBA, and ESBL (Biomerieux®) media. The concordance rate was calculated as the proportion of concordant pairs over the sum of concordant and discordant pairs. In case of discordant results, patients were kept in isolation for up to 3 consecutive culture-negative rectal swabs.

Results

Out of 1630 rectal swabs, 63 (3.9%) were positive for at least one CRE gene by MA. In particular, 28.5% KPC (n=18), 25.5% NDM (16), 19% VIM (12), 8% OXA48 (5), 6% KPC+NDM (4), 6% KPC+NDM+VIM (4), 3% NDM+VIM (2), 2% OXA48+VIM (1) and 2% OXA48+NDM (1) were detected.

The CA confirmed positivity in 48 samples (76.2%). The concordance rate (+/+) between molecular and conventional approaches was the following: 83.5% KPC (15/18), 87.5% NDM (14/16), 41.5% VIM (5/12), 80% OXA48 (4/5), 100% OXA48+NDM (1/1), 100% OXA48+VIM (1/1), 100% KPC+NDM (4/4) and 100% KPC+NDM+VIM (4/4). Good concordance rates were obtained between the two methods, except for VIM with over 50% of rectal swabs being culture-negative.

Results were discordant (+/-) for 15 samples. Of note, in none of the 3 follow-up swabs, the resistance mechanism detected by the MA was confirmed by CA.

Of the 87 rectal swabs tested invalid by MA (5.4%), 63 (72.0%) were negative by CA and 24 (28.0%) were defined as unsuitable samples because growth was absent on bacterial media.

Discussion and conclusions

The application of a molecular approach has been confirmed to be an accurate, useful, and fast method for the early detection of CRE genes among clinical isolates. A lower concordance between MA and CA was obtained only for VIM, requiring further investigations to define its source. Overall, these results strengthen the role of fast microbiology to aid in rapid surveillance, improve patient care, and enhance clinical workflow including antimicrobial stewardship.

28 - WHOLE GENOME SEQUENCING UNVEILS A MULTIDRUG RESISTANT P. AERUGINOSA ST2940 PNEUMONIA OUTBREAK IN AN INTENSIVE CARE UNIT IN NORTHERN LOMBARDY

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Whole genome sequencing unveils a multidrug resistant P. aeruginosa ST2940 pneumonia outbreak in an intensive care unit in Northern Lombardy.

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Introduction. We describe the characterization of an intensive care unit (ICU) outbreak of Pseudomonas aeruginosa, at Fondazione IRCCS San Gerardo dei Tintori (Monza - Italy) during summer 2023. From May to July 2023, 13 patients mechanically ventilated with clinical signs of pneumonia underwent lung fibroscopy for microbiological evaluation. As far as we noticed an increase of P.aeruginosa isolation trend, several samples from environment and clinical devices were also screened as potential source of the pathogen. Materials and Methods. Coltural tests from broncho-alveolar lavage and environment samples were performed on selective media. Colonies were identified by MALDI-TOF and tested for antibiotic susceptibility following EUCAST indications. Whole genome sequencing (WGS) was performed using Nextera DNA Prep kit (Illumina) following manufacturer protocol and sequenced on Illumina MySeq. Raw reads were checked for quality metrics, de novo assembly, genome annotation and downstream bioinformatic analisys were performed; in particular, we used Fastp (v. 0.23.4) for quality checking, SPAdes (v. 3.15.4) for de novo assembling and ABRicate (https://github.com/tseemann/abricate, accessed on 24 April 2024) to compare AMR genes, virulence and plasmid replicon. Pangenome analysis was performed with Prokka (v. 1.14.5) and Roary stand-alone pan genome pipeline. A maximum-likelihood (ML) phylogenetic tree was generated on the core genome alignment by iqtree (v. 2.2.2.6) and visualized using iTol (v. 6.6) web server (https://itol.embl.de/, accessed on 24 April 2024). Results. Phenotypic evaluation of 13 strains of P.aeruginosa retrieved from patients samples showed identical antibiotic resistance pattern as 3 strains grown from sample of washing liquids of reusable fiberscopes. All strains were resistant to carbapenems and chinolons, but not to amikacin, colistin and protected IV generation cephalosporins. WGS was performed on 11 strains from patients and 3 from devices. Multilocus sequence type identified all strains as ST2940. Pangenome analisys identified genes for resistance to lactams (blaPDC-374, blaOXA-494), aminoglicosides (aph(3')-Iib), fosfomycin (fosA), tetracyclines (tmexD2) and chloramphenicol (catB7) and multiple virulence factors. Discussion and Conclusions.

Pangenome and phylogenetic analisys showed a strict similarity between devices and patients strains gathering the evidence of monoclonal origin of P.aeruginosa pneumonia outbreak, maybe linked to reusable fiberscopes. On august 2023 all fiberscopes were sterilized by ethilen oxide and disposable fiberscopes were also made available to the ICU: after the interventions no further isolates of P.aeruginosa were found neither in patients nor in reusable devices.

38 - DIAGNOSIS OF A PERIPROSTETIC INFECTION SUSTAINED BY MYCOBACTERIUM FORTUITUM

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Diagnosis of a periprostetic infection sustained by Mycobacterium fortuitum

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Introduction

Non Tubercular Mycobacteria (NTM) are being isolated with increasing frequency from wound infections after surgery or trauma.The Mycobacterium fortuitum group is responsible for the majority of cases of postsurgical wound infections and catheter infections caused by the rapidly growing mycobacteria. We hereby present the case occurred in our University hospital “Policlinico Umberto I” of breast prosthetic infection by M. fortuitum and post tuberous breast deformity correction in a 21 years old woman. The infection became clinically evident one month after surgery.

Materials and methods

The first sample received in our lab was a breast seroma that was incubated in brain heart infusion broth for 24 hours at 37°C. After 12 days, we received periprosthetic secretion inserted in Bact-ALERT bottles (bioMérieux, Italy) for culture of aerobic bacteria and anaerobic bacteria, which were incubated in BACT/ALERT VIRTUO (bioMérieux, Italy). The bottles resulted positive after 48 hours of incubation. All samples were thereafter subcultured on columbia blood agar (COL), Mac Conkey agar, mannitol salt agar and incubated for 48 hours at 37°C. The MTB/NTM panel SD Biosensor (SD Biosensor, Republic of Korea) was used to reveal the IS6110 region for MTB and ITS region for NTM. NTM identification was performed with subculture on NTM Elite agar (bioMiérieux, Italy) and by mass spectrometry using a MALDI-TOF Biotyper (Bruker Daltonics Inc., Germany).

Results

In the COL and NTM subcultures colonies with smooth, rounded, creamy-white margins were observed. Despite its optimal use is with human normal sputum or sputum sediment specimens, colonies harvested from the agar plates sample were diluted in M10 sputum pretreatment Kit (SD Biosensor, Republic of Korea) and utilized for the identification of NTM using the SD BIOSENSOR

MTB/NTM, which revealed the presence of the ITS region after 18 cycles. MALDI-TOF performed on the isolated colonies grown on NTM Elite agar, gave the final M. fortuitum species identification on each tested sample. The strain tested multidrug resistant and susceptible to tigecycline.

Discussion and Conclusions

The diagnostic approach followed in this clinical case benefitted on the use of biomolecular methods in the fast recognition of NTM, and the use of specific culture media allowed the identification by MALDI-TOF. Therefore, the implementation of various techniques may be instrumental to define the aetiology of atypical mycobacteria. Evaluating the pathogenic potential of an isolate relies on various factors, including the site of isolation, frequency of isolation, patient's condition, and medical history. Most important of all is close liaison between clinician and laboratory.

67 - AUTOMIZED ANTIMICROBIAL SUSCEPTIBILITY TESTING AND INTERPRETATION BY DISC DIFFUSION METHOD: AN OLD APPROACH WITH A RENEWAL EFFICIENCY

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Automized antimicrobial susceptibility testing and interpretation by disc diffusion method: an old approach with a renewal efficiency

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Introduction: Sepsis and bacteremia are two severe clinical conditions that require a prompt identification of the infecting bacterium and a rapid Antimicrobial Susceptibility Testing (AST) result. The testing workflow and the speed of results are important, especially if the laboratory is centralized as in our setting. This study aims to define the impact of the recent introduction of a fully automated system for rapid AST performed by disc diffusion method directly on blood culture bottles, Radian® (Copan Group, Brescia, Italy). Material and Methods: 34 blood cultures positive for E. coli were included in the study. At first, a Gram-stained smear and a Columbia agar + 5% sheep blood (COS) plate were arranged for microscopic and MALDI-TOF identification. Then, the blood culture was diluted 1:3 in saline solution, and tested on Radian®. Samples were automatically streaked and disks were applied on the plate thanks to Radian® in-line carousel. Plates were then incubated and photos were automatically acquired after 4, 6, 8, and 18 hours. Inhibition zones were automatically measured and interpreted by the Radian® Expert System according to EUCAST's 2023 guidelines. Results were compared with a broth microdilution method currently used routinely in our lab. Results: for the 34 blood cultures, the average time between the Time-to-Positivity and the unloading time of the samples was evaluated in about 9 hours. Then, the time needed for transport and identification is about 6 hours. The average time to have a readable inhibition zone in Radian plates is about 5h. Considering the processing time, the Radian® workflow takes about 7 hours to evaluate a complete phenotypic susceptibility profile, whereas the broth microdilution method takes about 43 hours. The Category

Agreement between the two methods resulted in 98% (242/247) with 2% (5/247) of Minor Errors. Discussion and Conclusion: The introduction of Radian not only reduces variable costs for AST but also accelerates the turnaround time (TAT) for obtaining a susceptibility profile of the bacteria causing the infection, thus enhancing efficiency. Moreover, the method demonstrates high accuracy, underscoring its reliability and potentiality to streamline the diagnostic processes in infectious disease management.

68 - DEVELOPMENT OF RAPID METHOD FOR DETECTING VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) RESISTANCE USING ALFRED60/AST (ALIFAX)

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Development of rapid method for detecting vancomycin-resistant enterococci (VRE) resistance using Alfred60/AST (Alifax)

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Introduction: Vancomycin-resistant enterococci (VRE) has emerged in the last decade resulting in an increasing number of nosocomial infections. Enterococci are Gram-positive bacteria that are part of the normal intestinal flora and tend to cause infections only when the patient becomes frail as a result of other infections, diseases, or drug treatments. ALFRED60/AST is an automated system based on Laser Light Scattering technology (Alifax s.r.l.) which is able to detect microbial growth in vials containing liquid culture media. The Light Scattering system can be used for different applications when used in combination with specific reagents, such as Urine microbiological screening, Residual Antimicrobial Activity (R.A.A.), Antimicrobial Susceptibility Testing (AST) and MDRO screening. The evaluation of Alfred60/AST (Alifax s.r.l) for the application of VRE screening method from rectal swabs aims to provide phenotypic results to identify carriers of VRE. Materials and Methods: Using ALFRED 60/AST, a comparison was conducted between the prototype HB&L VRE kit (Alifax S.r.l.) for rectal swabs and the standard practice of seeding on selective chromogenic agar (bioMérieux®), the latter being used as a reference method. In March 2024, at the Greater Romagna Area Hub Laboratory (Cesena, Italy), 230 samples were collected, of which 29 were excluded because they were unsuitable and 14 were excluded due to doubtful results in the reference test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated on the remaining 187 samples. The analysis was performed using a selective liquid culture obtained by adding a lyophilized supplement to a liquid culture medium (prototype of HB&L VRE kit). The initial sample consisted of 200 µl of liquid from a fecal swab. The average analysis time was approximately 7 hours. Results: From the results obtained from the analysis of 187 rectal swabs, using the new VRE prototype kit from HB&L (Alifax S.r.l.), the sensitivity and VVP (Predictive Positive Value) were 87%, while the specificity and VPN (Predictive Negative Value) were 94%. Discussion and Conclusions: Based on the data obtained from the first trials conducted on the prototype under development, some significant preliminary conclusions can be drawn. Currently, the process is being optimized on several key aspects, including the composition of the supplement, the time required for analysis and the algorithm used for detecting

positivity. Once this optimization phase is completed, more accurate results and reduced analysis time are expected, thus contributing to improved screening diagnosis of VRE.

78 - CALPROTECTIN AS A NOVEL BIOMARKER OF SEPSIS AMONG INFLAMMATORY CASCADE MEDIATORS MEASURED IN CRITICAL PATIENTS

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Calprotectin as a novel biomarker of sepsis among inflammatory cascade mediators measured in critical patients.

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Introduction. Sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to systemic infection, is a major public health problem with a high mortality and morbidity rate. The high complexity of the pathophysiology of sepsis requires further investigation to determine the immune response in sepsis and septic shock and many biomarkers implicated in clinical practice are not sufficiently specific to differentiate sepsis from other non-infectious inflammatory disorders. Therefore, the aim of this observational retrospective study is to evaluate more specific serum biomarkers, such as pro-inflammatory cytokines and serum-calprotectin in patients with sepsis and septic shock, to help clinicians to diagnosticate and prognosticate patient death risk.

Material and Methods. For this study, 57 patients (aged 18 or older), who were subjected to blood culture testing for the purpose of diagnosing sepsis, were enrolled. Studied subjects were divided into three groups: 15 healthy volunteers (control group), 15 patients exhibiting SIRS but with negative blood culture (SIRS group), 42 patients with positive blood culture for gram-negative bacteria (septic group). Patients underwent evaluation of pro-inflammatory and anti-inflammatory cytokines and serum-calprotectin. Cytokines levels were assessed on serum samples by biochip array technology (chemiluminescence, kit CTK HS – Randox), while samples of serum-calprotectin were evaluated by immunoenzymatic assay (ELISA test, kit Calprest NG – Eurospital Diagnostic).

Results. Calprotectin levels evaluated on human septic serum samples showed a significant ($p<0.05$) difference between both septic versus controls and septic versus SIRS values. TNF-alpha septic samples exhibited significant ($p<0.05$) higher level versus both controls and SIRS group. On the contrary, IL-4 did not show any significant differences among groups studied. Concentration of IL-10 in

septic group, exhibited significant ($p<0.05$) higher levels versus both controls and SIRS group. Values of IL-6 showed an even more interesting pattern, because a significant ($p<0.05$) difference between septic samples versus controls was associated with a significant difference between SIRS and septic samples. A typical neutrophil chemokine IL-8 again exhibited a significant ($p<0.05$) difference between septic group and controls group and also between septic and SIRS group. Based on our data, calprotectin, IL-6 and IL-8, showed the same interesting behaviour.

Discussion and Conclusions. In conclusion, calprotectin as a novel biomarker might be helpful for the clinicians to distinguish between sepsis and SIRS, as well as to estimate the prognosis among very critically patients, a long time before the availability of the blood culture results.

83 - CHARACTERIZATION OF MICROORGANISMS ACCORDING TO VAGINAL COMMUNITY STATE TYPES MICROBIOTA: AN OBSERVATIONAL STUDY AMONG WOMEN ACROSS ITALY

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Characterization of microorganisms according to vaginal community state types microbiota: an observational study among women across Italy

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Introduction: The vaginal Microbiome and composition of microbial flora play an important role in maintaining female health, and it's fluctuating during the women's entire life. The aim of this work is to characterize microbial composition and dysbiosis level in a large population of healthy, paucisymtomatic or symptomatic women.

Methods: Community State Type (CST) was assigned by a Lactobacilli Flora Genotyping Realtime PCR (RT-PCR, KIT vaginitis and vaginosis by AUS Diagnostic) and an in-house informatic Pipeline; other pathogens were detected by a commercial RT-PCR (Seegene, Alloplex Kits). Dysbiosis level according to CST was categorized in 3 groups: (i) Normal (N): CST I or CST II; (ii) partially dysbiotic (PD): CST III or CST V; (iii) dysbiotic (D): CST IV. Covariation analyses were performed to explore potential microrganisms interactions. Regression models (RM) were used to evaluate predictors of dysbiosis (D or PD) according to demographic, physiological and microbial detection.

Results: Overall, 203 women with a median (IQR) age of 39 (34-55) were analysed. Of them, 50.2% were symptomatic, 70.4% at the reproductive state, 8.4% in menopause and 72.9% with normal BMI. Most individuals showed N level (48.8%), followed by PD (35.0%) and D (16.2%). At increasing dysbiosis level an older age was observed (D vs. PD vs. N: 44 [38-68] vs. 41 [34-51] vs. 37 [31-52], $p<0.001$). The most frequent microorganisms were Gardnerella vaginalis (50.7%), Atopobium vaginae (33.5%), Candida albicans (24.6%); Ureaplasma parvum (23.6%) and Streptococcus agalactiae (19.7%). In about half of women (53.7%), ≥2 microorganisms were detected. Dysbiosis (PD or D levels) was significantly associated with the number of microorganisms detected (0, 1, ≥2: 35.3%, 41.9%, 62.4%, P=0.002). Compared to dysbiotic (PD or D level), in individuals with normal flora, Gardnerella vaginalis (41.7% vs. 58.3%, P=0.042), Ureaplasma parvum (35.4% vs. 64.6%, P=0.034), Atopobium vaginae (39.7% vs. 60.3%, P=0.067) and Candida albicans (19.2% vs. 29.8%, P=0.079) were not frequent. As expected, microorganisms were often associated to each other. However, the pathogenic Streptococcus agalactiae significantly correlated only with Candida albicans, with a correlation

frequency of 42.5% (Phi=0.205, P=0.003). According to RM, older age (per 5-years increase, OR 95 C.I.: 1.4 [1.2-1.7], P<0.001) and presence of Candida albicans (2.3 [1.1-4.6] P=0.019) independently predicted dysbiosis.

Discussion and Conclusions: In this observational study, about half of individuals showed normal vaginal microbiota. Older age and the presence of multiple microorganisms, in particular Candida albicans were predictive of dysbiotic vaginal flora.

91 - DIAGNOSTIC ROLE OF CALPROTECTIN IN BRONCHOALVEOLAR LAVAGE SAMPLES FOR EARLY PNEUMONIA DIAGNOSIS

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Diagnostic role of calprotectin in bronchoalveolar lavage samples for early pneumonia diagnosis

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Introduction: The role of biomarkers in diagnosis of Community-Acquired Pneumonia (CAP) and Ventilator-Associated Pneumonia (VAP) infections is yet to be investigated. The evaluation of calprotectin (CLP) role in the pathogenesis of respiratory diseases and its usefulness as a biomarker for the appropriate diagnosis and prognosis of lung diseases have only gained attention in recent years. CLP is a multifunctional protein expressed mainly by neutrophils. This protein is extracellularly released by activated or damaged cells subsequently to broad range of physiological and pathological responses. Calprotectin concentrations in serum and stools have been investigated for diagnosis of acute respiratory infections, but CLP in bronchoalveolar lavage (BAL). has not been reported yet. In the present study, we aimed to evaluate the CLP level in BAL samples for timely prediction of pneumonia, suggesting the usefulness of this biomarker in clinical practice. Materials and Methods: Fifty-two BAL from 34 patients with symptoms of respiratory infections (study group) and 18 asymptomatic patients (control group) were included. Quantitative determination of CLP was assessed by chemiluminescence sandwich method (Liaison Calprotectin fecal assay, Diasorin) modified for bronchoalveolar samples (off-label). A cut-off value of > 50 µg/ml was considered positive. The diagnosis of low respiratory tract infection was supported by positive bacterial cultures or multiplex-PCR test (FilmArray Pneumonia Panel, bioMérieux). Results: Patients of study group were infected by bacteria (15/34), viruses (7/34) or mixed infections (12/34). We found higher levels of CLP in patients with respiratory infections in comparison to control group (<50 µg/ml). Moreover, higher CLP values in patients with bacterial respiratory compared to patients with viral respiratory infections were detected. A CLP level ranging from 50 to 150µg/ml was found in patients with viral respiratory infection, while CLP levels >150µg/ml were frequently detected for bacterial disease. In addition, higher concentrations of CLP were found in patients with mixed infections compared to patients with viral or bacterial lower respiratory tract infections. Discussion and Conclusions: Rapid pneumonia diagnosis is mandatory. CLP values <50µg/ml could exclude a respiratory infection. Moreover, CLP

was able to distinguish between bacterial and viral infections allowing a timely and presumptive diagnosis. Even if further investigation is necessary, our preliminary data suggest a key role of CLP in early pneumonia diagnosis when evaluated in BAL samples. Fecal CLP assay adapted for bronchoalveolar lavage samples showed adequate technical performances. A diagnostic algorithm is under process.

94 - USING T2MR TECHNOLOGY IN CRITICALLY ILL SEPTIC PATIENTS: ARE WE OVERCOMING THE LIMITS OF BLOOD CULTURE?

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Using T2MR technology in critically ill septic patients: are we overcoming the limits of blood culture?

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Introduction. Bloodstream infections (BSI) are among the most common life-threatening infections. Rapid and accurate detection and identification of BSI causative agent and characterization of its antimicrobial susceptibility are crucial in reducing mortality and morbidity rates. However, the long turn-around time (TAT) of blood culture (BC), the gold standard for BSI diagnosis, is a major drawback in the management of severely hill septic patients. The T2MR technology, FDA cleared, is a novel molecular assay for the identification of ESKAPE bacteria and five Candida spp directly in blood specimens, which can help to overcome BC long TAT. We analysed the preliminary results obtained in critically ill patients at Padua's University Hospital between November 2023 and March 2024 using the T2MR technology to evaluate its performance with respect to BC.

Materials and Methods. During the study period, for the purpose of T2MR processing, whole blood was obtained from thirty-nine patients with clinical and laboratory signs of severe sepsis. Eight were analysed with T2Bacteria, four with T2Candida, and twenty-seven with both panels, and results compared to simultaneously collected BC and any culture from relevant body fluids performed within 14 days.

Results. Majority of requests were from intensive care units (17/39) and paediatric haemato-oncology (15/39), whereas only six requests were from clinical wards and one from the Emergency room. All patients, except the one recruited at the ER, were receiving broad-spectrum antibiotics for >48 hours at the sampling time. BC resulted positive in 3/39 (7.7%) of patients: in one case T2 and BC identified the same microorganism (P. aeruginosa) [proven BSI] whereas T2 was negative in two positive BC since no ESKAPE microorganisms were detected (S. maltophilia and S. mitis). Among the 36 patients with negative BC, identical results were obtained in 30 T2 assays. In six patients with negative BC, T2 provided a positive result: two cases were probable BSI (E. faecium, P. aeruginosa and C. albicans identified with T2 were detected within 14 days in bile and abdominal drainage fluid), whereas four cases were possible BSI (positive T2Bacteria or Candida Panel in the absence of supporting culture data within 14 days). Taking into account positive BC as the gold-standard for BSI diagnosis, T2MR technology showed a 100% sensitivity with a 83% specificity.

Discussion and Conclusions. T2Bacteria and Candida Panels evidenced high sensitivity in septic patients providing reliable support to confirm or exclude ESKAPE BSIs in critically ill patients. A larger study is necessary to determine the exact clinical impact of T2Bacteria and Candida Panel results on length of hospital stay and mortality benefit in critically ill patients with suspected BSI.

98 - THE CONTRIBUTION OF MOLECULAR BIOLOGY IN THE DIAGNOSIS OF BACTERIAL GASTROENTERITIS

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The contribution of molecular biology in the diagnosis of bacterial gastroenteritis

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Introduction Gastrointestinal infections are serious global health problem but their impact is still often underestimated. The term gastroenteritis describes an inflammation of the stomach and intestine due to viruses about 70%, bacteria about 30% and a small percentage by fungi, parasites or non-infectious causes. Furthermore, the clinical presentation is not usually indicative of a specific pathogen. Rapid and accurate detection of the etiological agent is important for appropriate therapy and infection control. The aim of this study was to evaluate the contribution of molecular biology in diagnosis of bacterial gastroenteritis by comparing the results obtained with reference culture method. Materials and Methods The stool samples under examination were first subjected to culture examination by seeding on selective media for the detection of the main bacterial pathogens involved in gastrointestinal infections, with readings at 24 hours and at 48 hours after reseeding from enrichment broth. The bacteria were identified by MALDI-TOF (VITEK® MS PRIME, Biomerieux, Francia). The samples were stored at -41°C pending molecular analysis. Bacterial DNA was extracted by Microlab Starlet IVD instrument (Hamilton, Nevada, USA). DNA amplification was performed by CFX96™ real-time PCR system (Biorad, California, USA) and carried out using Allplex™ GI-Bacteria(I) Assay (Seegene, Seoul, South Korea). Seegene Viewer Software (Seegene Inc. Seoul, Korea) was used for detection and data analysis. Results From April to September 2023, 125 faecal samples were examined. Molecular analysis showed 48 positive sample (38%) versus 40 (32%) by culture method. Particularly, molecular assay detected 20 Campylobacter spp. (41.7%), 15 Salmonella spp. (31.2%), 6 Areomonas spp. (12.5%) and 2 Shigella spp. (4.2%). On the other hand, reference method identified 20 Campylobacter spp. (50%), 18 Salmonella spp. (45%), 1 Areomonas spp. (2.5%) and 1 Shigella spp. (2.5%).

Analysis of discordant results showed that the molecular assay detected 8 pathogens that the culture did not detect which included 2 Campylobacter spp. (25%), 5 Areomonas spp. (62.5%) and 1 Shigella spp. (12.5%). In addition, molecular analysis detected 5 co-infections. Discussion and Conclusion The use of molecular assay has made it possible to identify bacteria that are difficult to isolate using conventional culture methods, albeit with some limitations related to possible false positivity. All in all, we can conclude that molecular biology can be extremely useful when combined with conventional diagnostic methods, especially for rapidly reporting negative samples.

110 - TITLE: INVESTIGATING THE DYNAMICS OF MRSA AND MSSA COEXISTENCE IN CYSTIC FIBROSIS PATIENTS: FOURIER-TRANSFORM INFRARED SPECTROSCOPY AS AN ALTERNATIVE TO WGS FOR STRAIN TYPING

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APPROCCI DIAGNOSTICI

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Title: Investigating the dynamics of MRSA and MSSA coexistence in cystic fibrosis patients: Fourier-transform infrared spectroscopy as an alternative to WGS for strain typing

Introduction: Staphylococcus aureus is one of the main pathogens colonizing the airways of cystic fibrosis (CF) patients, and the surveillance of this pathogen for the screening of methicillin resistance is crucial for guiding therapy decisions. Methicillin-Sensitive S. aureus (MSSA) is spread in children and is then usually replaced by Methicillin-resistant S. aureus (MRSA). The mechanisms underlying this replacement from MSSA to MRSA are still under investigation, in particular to understand whether the emergence of resistance is given by the acquisition of new strains or by the acquisition of resistance by another MRSA. To address this, we propose a pilot study designed to explore the potential of Fourier Transform Infrared Spectroscopy (FTIR) as an alternative to Whole Genome Sequencing (WGS) for strain typing. We identified three CF patients colonized by both MSSA and MRSA, aiming to evaluate the clonality of these strains.

Materials and Methods: We identified two morphologically distinct colonies of S. aureus in clinical samples from three CF patients and screened them for methicillin resistance using Oxacillin and Cefoxitin disc diffusion tests. Methicillin resistance was confirmed through subculture on MRSA agar and penicillin-binding protein 2a (PBP2a) testing. Antibiotic susceptibility was tested, and strain characterization was performed with FTIR and WGS. FTIR spectra were analyzed with the IR Biotyper, through hierarchical clustering Principal Component Analysis (PCA), in order to evaluate the degree of genomic relatedness among pairs of isolates.

Results: Hierarchical clustering and PCA analysis revealed no correlation between MRSA and MSSA strain pairs in all patients, suggesting that they belonged to different sequence types. WGS confirmed

these findings revealing that the MRSA and MSSA strain pairs were not clones. Specifically, the strain pairs belonged to different STs and displayed different spa types. Regarding the methicillin resistance, two strains were found to carry the type IV (2B) Staphylococcal Cassette Chromosome mec (SCCmec), while one the type 1 (1B). Moreover, WGS revealed the presence of Panton-Valentine Leukocidin virulence factor in 4 strains, namely 3 MRSA and 1 MSSA.

Discussion and Conclusions: Our analysis suggests that coexistence of MRSA and MSSA strains in our CF patients derived from the acquisition of a MRSA clone rather than the acquisition of resistance determinants by the MSSA already colonizing the patient. Additionally, the agreement between FTIR and WGS underscores FTIR's potential as a predictive tool. In fact, the FTIR represents a rapid and cost effective tool for the screening of this pathogen, in order to better understand the correlation between resistance and clones in our patients.

111 - INTEGRATING DIAGNOSTIC APPROACHES IN BACTERIAL MENINGITIS CAUSED BY A NON-K1 ESCHERICHIA COLI: A CLINICAL EXPERIENCE

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Title: Integrating diagnostic approaches in bacterial meningitis caused by a non-K1 Escherichia coli: a clinical experience

Introduction: The FilmArray Meningitis Panel BioFire is a valuable tool for diagnosis of meningitis caused by various pathogens, including Escherichia coli K1, and is the only panel certified for cerebrospinal fluid (CSF). This syndromic panel is able to identify 14 pathogens in 1 hour, but not antimicrobial resistance (AMR) mechanisms. However, its exclusive targeting of the K1 antigen may lead to misdiagnosis of meningitis caused by non-K1 E. coli strains. We present a clinical case of a seven-month-old child with suspected meningitis initially managed at a local hospital before being transferred to our center. The negative result from the FilmArray Meningitis Panel on CSF delayed the identification and treatment of a non-K1 E. coli strain bearing CTX-M-15, ultimately resulting in patient's death.

Materials and Methods: CSF sample was first analyzed using FilmArray meningitis BioFire. After chemical and physical analyses were conducted to assess metabolic abnormalities, the FilmArray Blood Culture Identification (BCID) Panel was used off label on the CSF sample. The sample was subcultured on agar plates. Whole genome sequencing (WGS) was then performed after DNA extraction of the microorganism grown on Blood Agar to characterize the strain and study AMR and virulence.

Results: While the FilmArray Meningitis Panel yielded a negative result, glucose consumption in the CSF, suggestive of a bacterial infection, was detected via chemical analysis. A FilmArray BCID Panel was thus performed, identifying the presence of a non-K1 Escherichia coli strain carrying CTX-M. WGS analysis confirmed the presence of the E. coli ST131 strain, serotype O25:H4 fumC40/fimH30, a resistant high-risk clone often associated with sepsis and urinary tract infections. Regarding AMR, the strain carried several genes, including CTX-M-15 and TEM-1B, and gyrA and parC mutations associated

to fluoroquinolones resistance. Regarding virulence, the strain carried several virulence factors, including a Conserved Virulence Plasmidic (CVP) region, already linked to neonatal meningitidis cases due to non-K1 E. coli.

Discussion and Conclusions: The syndromic panel used in our laboratory routine did not allow the identification of non-K1 E. coli strain carrying CTX-M due to target absence. The delay in the modification of an adequate empirical therapy ultimately led to the death of the patient, even though the virulence of this high-risk clone could have played the greatest role in the progression to a fatal outcome.This case underscores the importance of integrating different approaches in the diagnosis of bacterial meningitis. We propose a workflow involving both infectious disease specialists and microbiologists to synergistically interpret test results and guide therapeutic decisions.

112 - IDENTIFICATION OF USTILAGO SPP INFECTION IN A PEDIATRIC PATIENT UNDERGOING OPEN-CHEST EXTRACORPOREAL MEMBRANE OXYGENATION THROUGH WHOLE GENOME SEQUENCING

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Title: Identification of Ustilago spp infection in a pediatric patient undergoing open-chest extracorporeal membrane oxygenation through Whole Genome Sequencing

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Introduction:

Immunocompromised patients, particularly those undergoing extracorporeal membrane oxygenation (ECMO), are susceptible to nosocomial infections, including fungal diseases. While Candida and Aspergillus species are commonly implicated, other fungal pathogens are emerging. We present a case of a 3-year-old male, born preterm, with history of pulmonary hypertension, and bronchopulmonary dysplasia, who was hospitalized for respiratory failure during influenza A and RSV infection and required open-chest ECMO support. Due to radiological worsening and high risk of fungal infection, tests for identification of these microorganisms were requested. After standard methods and molecular techniques failed to identify the cause of infection, Whole Genome Sequencing (WGS) revealed the presence of a fungus belonging to the Ustilago genus, a group of fungi commonly found in the environment, but rarely causing human diseases.

Materials and Methods: Whole blood samples and blood cultures were sent to our laboratory, and the latter were used for T2Candida panel (T2 Biosystems). Blood cultures resulted positive after 4 days and positivity for fungi was confirmed by Gram staining. The blood cultures were then plated on agar media and the microorganism was isolated from blood agar and Sabouraud dextrose agar. The fungus underwent typing using Bruker MALDI-TOF MS System and Autobio Autof ms1000. WGS was finally performed on both blood and suspension samples.

Results: Although the T2Candida panel returned negative, empirical antifungal treatment with caspofungin was started due to positive findings from blood cultures. No identification was obtained by mass spectrometry. Notably, the fungus exhibited a mucoid phenotype on Sabouraud dextrose agar. Despite caspofungin treatment, there was no improvement in the patient's conditions. Finally, WGS analysis, coupled with phylogenetic analysis based on Universal Fungal Core Genes, identified the presence of a species belonging to Ustilago genus. Despite the paucity of literature describing treatments for Ustilago spp infections, adjustements to therapy, including the addition of ambisome and voriconazole, led to a positive response and eradication of the Ustilago spp.

Discussion and Conclusions: This case underscores the importance of correct identification of fungal infections in immunocompromised patients and emphasizes the need for research addressing emerging fungal pathogens. It also highlights the importance of advanced molecular techniques like WGS in the identification of these microorganisms, allowing for correct diagnosis and improved patient care. Moreover, following WGS identification, we acquired the mass spectrometry spectrum in order to allow future identification of this pathogen.

138 - MTBC/NTM DETECTION AND DISCRIMINATION: A NEW RAPID MOLECULAR ASSAY EVALUATION

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Topic: Approcci diagnostici

MTBC/NTM detection and discrimination: a new rapid molecular assay evaluation

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Introduction

According to WHO guidelines, the diagnostic workflow for Mycobacterium tuberculosis complex (MTBC) and Non-Tuberculous Mycobacteria (NTM) detection in clinical sample is based on 3 steps: microscopy, culture with liquid and solid media and nucleic acid amplification tests (NAATs) for MTBC. Microscopy is a rapid test, but its sensitivity is low, and it is unable to distinguish between MTBC and NTM. Culture is the gold standard diagnostic test for mycobacteria detection unless turn-around time (TAT) is of days or weeks. Rapid molecular tests for mycobacteria detection in clinical samples could solve TAT issue. For this reason, we assessed the performance of the new molecular assay STANDARD M10 MTB/NTM (M10) (SD BIOSENSOR, Republic of Korea) for rapid MTBC and NTM detection and discrimination.

Materials and Methods

78 samples, 81% respiratory and 19% non-respiratory (19%), NALC/NaOH decontaminated and thermically inactivated samples were stored and subsequently tested with M10 according to manufacturer instructions. Briefly, samples were incubated with phosphate/H2O buffer for 15 minutes, then 1.4 mL of sample loaded into the cartridge with expected results within 72 minutes. The M10 results were evaluated in comparison with fluorescent microscopy, Xpert MTB/Rif Ultra (Ultra) (Cepheid, USA), and culture results available from routinary laboratory activity.

Results

No invalid results were obtained. Considering microscopy, the concordance was 77% with 7% M10+/microscopy- and 15% M10-/microscopy+ (all microscopic scanty results). Concerning molecular assay, the agreement between MTB Ultra and MTB M10 detection was 89% with 11% M10-/Ultra+. On culture, the concordance was 67% with 30% M10-/culture+ (29% MTBC) and 2% M10+/culture- (1 patient on antimicrobial NTM treatment). 41% of M10 positive results were available before 72 minutes scheduled of test (88% MTB, 12% NTM).

Conclusions

The comparison data confirm the reliability of M10 results and underline the possibility to rapidly detect and discriminate MTB and NTM from different clinical samples. Moreover, in 7% (50% MTB and 50% NTM+) of the cases, M10 provided positive results, in otherwise microscopy negative samples, with a significant gain in terms of TAT in comparison with conventional methods. Finally, the agreement between M10 and culture confirm the important role of culture as gold standard diagnostic test in mycobacteria infections.

139 - COMPARISON BETWEEN MOLECULAR, MASS SPECTROMETRY AND PHENOTYPIC METHODS FOR RAPID DETECTION OF METHICILLIN-RESISTANCE AND PVL GENES IN STAPHYLOCOCCUS AUREUS CLINICAL ISOLATES

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Topic: Approcci diagnostici

Circa 2357/2800 caratteri spazi inclusi (no autori e affiliazioni)

Comparison between molecular, mass spectrometry and phenotypic methods for rapid detection of methicillin-resistance and PVL genes in Staphylococcus aureus clinical isolates

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Introduction

Staphylococcus aureus, a common multi-resistant bacterium, is cause of infection in different anatomical sites. The severity of the infection depends of S. aureus ability to produce virulence factors, such as cytotoxins α-δ and Panton-Valentine leucocidin (PVL), which also impact on antimicrobial treatment. The gold standard for antibiotic susceptibility testing is phenotypic antibiogram but its turn-around time (TAT), +24 hours from positive culture, could be too long for severe infections management, in particular with immunocompromised patients. Moreover, conventional methods are unable to detect virulent strains. In this study we evaluated a fast molecular assay for S. aureus identification, methicillin-resistance (MR) and PVL genes detection from isolated colonies.

Materials and Methods

Eighty-five strains of S. aureus selected and isolated from severe infections (70% respiratory), firstly were identified and screened for MR by Bruker microflex MALDI-TOF (Bruker, Germany), and after were tested with CE-IVD eazyplex® MRSAplus (eazyplex) (AmplexDiagnostic GmbH, Germany). Eazyplex is a nucleic acid isothermal amplification assay; it requires 2 minutes of sample pretreatment at 99°C and 15 minutes to results. Eazyplex S. aureus identification and detection of mecA/C and PVL genes were evaluated. MALDI-TOF identification and phenotypic antibiogram results by BD Phoenix PMIC/ID-88 (PH) (Becton Dickinson, USA) were used as reference (concordance and Fisher Exact were calculated).

Results

No invalid results were obtained. MALDI-TOF/PH MR concordance was 56% with 42% false negative and 2% false positive results . Eazyplex/MALDI-TOF identification agreement was 100% and Eazyplex/PH MR concordance was 99% with only one false positive. MR, identified with eazyplex, was associated only to mecA genes. Finally, 30% PVL-positive samples were identified, 64% were MR.

Conclusions

MR identification by MALDI-TOF could be an important diagnostic improvement, but its reliability is still poor. Eazyplex rapid and all-in-one solution for S. aureus identification and detection of mecA/C and PVL genes, could be an important tool in clinical microbiology due to its accuracy, low TAT and the provided information about virulent S. aureus strains, in order to initiate a prompt appropriate therapy.

142 - A ZETA POTENTIAL STUDY TO MEASURE S. AUREUS ADHESION ON HUMAN BONE OSTEOSARCOMA CELLS

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A zeta potential study to measure S. aureus adhesion on human bone osteosarcoma cells

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INTRODUCTION

A negative zeta potential keeps cells electrostatically repelled, maintaining structural stability. Different bacterial species have varying zeta potential values. This study is the first to examine how eukaryotic cells' zeta potential changes when in contact with a clinical isolate and the S. aureus ATCC USA300 strain. Previous studies have shown this phenomenon, and our goal is to track pathogen internalization through these variations.

MATERIALS AND METHODS

Human bone osteosarcoma cells (MG-63, CRL-1427TM)
were exposed to S. aureus USA300 strain at a multiplicity of infection (MOI) of 1:100 for two hours. Following this incubation period, non-adherent bacteria were removed through consecutive washes. In the case of the clinical isolate, cells were infected at three different time points (one, two, and three hours) to observe variations in zeta potential over time. Zeta potential values were determined using the Zetasizer Nano ZS90 (Malvern Panalytical) instrument. Both measurements and washes were performed using a custom buffer formulated in our laboratory with optimized biochemical and electrical properties.

RESULTS

The USA300 strain adhering to the cell surface drops the zeta potential from -20.4 mV to -25.7 mV (-25.98%), indicating the additive properties of zeta potential and induced system instability. Tukey's Multiple Comparisons Test shows a significant difference between untreated and infected cells ($P = 0.0006$). For the clinical strain, which hasn't established internalization, the bacterial basal zeta potential differs from USA300 (-19.3 mV). This value, close to MG-63's, results in a smaller zeta potential decrease upon adherence (-23.1 mV). At two hours, bacteria are adherent with the lowest zeta potential, returning to individual cell values at one and three hours. Both adherence conditions are statistically different from controls.

DISCUSSION and CONCLUSIONS

The study brings to light an additional dimension of destabilization induced by two strains of S. aureus, a pathogen widely acknowledged for its virulence, on eukaryotic cells. Specifically, the observed reduction in zeta potential precipitates a consequential shift in cell interactions. These findings underscore the intricate mechanisms at play during bacterial infections, particularly concerning their impact on cellular dynamics.

Furthermore, our results suggest the potential utility of this approach for both quantitative and qualitative evaluation of adhesion on eukaryotic cells. By leveraging this technique, researchers can gain deeper insights into the intricate interplay between pathogens and host cells, thereby facilitating the development of more effective therapeutic strategies.

161 - EVALUATION OF THE CLINICAL PERFORMANCE OF QIASTAT DX® BCID MOLECULAR SYSTEM FOR RAPID DIAGNOSTICS OF OF BLOODSTREAM INFECTIONS

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Evaluation of the clinical performance of QIAstat Dx® BCID molecular system for rapid diagnostics of of bloodstream infections

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Introduction

The QIAstat Dx® BCID (Qiagen) is a new automated system based on real-time multiplex PCR designed to detect main pathogens and antimicrobial resistance (AMR) genes directly from positive blood culture with a turnaround time of 70 minutes. This study evaluated the clinical performance of QIAstat Dx® using both clinical and spiked blood cultures (BC).

Material and Methods

The QIAstat Dx® GPF-Panel (Qiagen) is designed to detect 18 fungal and Gram-positive bacterial targets, 11 AMR genes and one pan gram-negative target. The QIAstat Dx® GN-Panel which is designed to detect 13 Gram-negative bacterial targets and 18 AMR genes. The two panels were used according to Gram-staining examination. Overall 198 clinical BCs (Gram-negative, n=98; Gram-positive, n= 88; fungi, n=12) and 43 spiked BCs (characterized MDR Gram-negative strains) were tested. Results obtained by molecular testing were compared with conventional results (MALDI-TOF identification, PCR assays for main β-lactamases genes, phenotypic AMR by automated microdilution system).

Results

Overall, QIAstat Dx® BCID panels identified microbial pathogens in 183 out of 198 BC samples. Fifteen samples (7.6%) were positive for bacterial species/genera not targeted by the assays. Excluding BC samples positive to not targeted pathogens, diagnostic sensitivity was 97.8%. The overall specificity was 98%.

AMR genes were detected in 89 out of 141 (63.1%) and in 49 out of 88 (55.7%) BC samples positive to Gram-negative and Gram-positive bacteria, respectively. Main carbapenemase genes (blaKPC, blaNDM, blaVIM, blaOXA-48-like, blaIMP, blaOXA-24/40, blaOXA-58) were detected in 56 out of the 130 BC samples positive to Enterobacterales or non-fermenting Gram-negative species, showing 100% agreement with conventional molecular testing. Other AMR genes detected by QIAstat Dx® GN-Panel included other beta-lactamases genes (SHV, TEM, CTX-M, VEB, and AmpC genes), aac(6')-1b and armA aminoglycoside resistance genes. Resistance genotype agreed with resistance phenotype in 97.7% of the Gram-negative isolates.

Among Gram-positive BCs, 51 out of 88 samples tested positive for AMR genes (mecA, blaZ, aac(6')aph(2'), vanA, vanB, tetK, tetM, ermA, ermC). Resistance genotype agreed with resistance phenotype in 97.7% of the Gram-positive isolates.

Discussion and conclusions

Based on this study, the QIAstat Dx® BCID panels represents a rapid and reliable test for the detection of common bloodstream pathogens and main AMR genes directly from BCs with a turnaround time of about one hour. Implementation of the system in blood cultures diagnostic workflow could enable optimal antimicrobial management of septic patients, especially in hospital setting with higher rates of multidrug resistance.

162 - DETECTION OF VOLATILE ORGANIC COMPOUNDS AS NEW PARADIGM TO ACCELERATE ANTIMICROBIAL SUSCEPTIBILITY TESTING

Gabriele Bianco *(1)* ***- Matteo Boattini*** *(1)* ***- Sara Comini*** *(1)* ***- Alessandro Bondi*** *(1)* ***- Antonio Curtoni*** *(1)* ***- Giorgia Piccinini*** *(1)* ***- Tiziana Musso*** *(1)* ***- Francesco Broccolo*** *(2)* ***- Rossana Cavallo*** *(1)* ***- Patrice Nordmann*** *(3)* ***- Cristina Costa*** *(1)*

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Detection of Volatile Organic Compounds as new paradigm to accelerate antimicrobial susceptibility testing

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Introduction

The measurement of VOCs release in the headspace of a bacterial culture represents a new approach to rapidly assess antimicrobial susceptibility. The VITEK® REVEAL™ technology is based on measurement of VOCs of bacteria combined with a selection of antibacterial agents by colorimetric sensor array which change color when exposed to different VOCs. Herein, we evaluated the diagnostic performance of the VITEK® REVEAL™ system directly from a collection of Gram-negative positive blood cultures.

Material and methods

Based on MALDI-TOF MS identification performed directly from clinical positive blood cultures, 91 BCs showing identification of Escherichia coli (n= 24), Klebsiella pneumoniae (n= 25), Pseudomonas aeruginosa (n= 16), Acinetobacter baumanni complex (n= 6), Enterobacter cloacae complex (n= 7), Proteus mirabilis (n=6), Citrobacter koseri (n= 3), Klebsiella aerogenes (n=2), Citrobacter freundi (n= 2) were selected and enrolled in the study. Additionally, 40 BCs spiked with molecular characterized Gram-negative clinical isolates recovered from Swiss National Reference Center for Emerging Antibiotic Resistance and from Italian hospitals. Samples were processed using VITEK® REVEAL™ according to the manufacturer's recommendations and MICs of twenty-two antimicrobials were compared with those obtained using standard broth microdilution. Categorical agreement (CA), essential agreement (EA), and categorical errors were calculated.

Results

Overall, 2220 strain/antibiotic pair combinations were analyzed. Of these, most were classified as resistant by reference AST (1091/2220, 48.7%). The overall CA and EA were 97.6% and 97.7%, respectively. CA ranged from 97.5% in Enterobacterales to 97.9% in both P. aeruginosa and A. baumannii complex. The overall number of categorical discrepancies were: 18 VME (1.6%), 13 ME (1.2%) and 22 mE (2.4%). EA ranged from 95.2% in P. aeruginosa to 98.1% in Enterobacterales. Screening test for ESBL phenotype was positive, indeterminate and negative in 13.7%, 32.6% and 27.4% of Enterobacterales isolates tested by both VITEK® REVEAL™ and reference method, showing 100% of CA.

Discussion and conclusions

VITEK® REVEAL™ represents a reliable tool to obtain antimicrobial susceptibility results of main Gram-negative species directly from positive BCs with a time-to-results of less than 8 hours.

169 - A NEW RAPID ANTIMICROBIAL SUSCEPTIBILITY TESTING ASSAY EVALUATED WITH POSITIVE BLOOD CULTURES FOR ANTIMICROBIAL-RESISTANT GRAM-NEGATIVE BACTERIA

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A new rapid antimicrobial susceptibility testing assay evaluated with positive blood cultures for antimicrobial-resistant gram-negative bacteria.

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Introduction

Early determination of antimicrobial susceptibility testing (AST) profiles for bacterial organisms that cause bloodstream infection is crucial to reduce the empirical use of broad-spectrum antibiotics. We evaluated a new rapid AST assay, the VITEK® REVEAL™ (bioMérieux) system, which detects the growth of Gram-negative (GN) bacteria via their emission of volatile organic compounds directly from positive blood cultures (PBCs).

Materials & Methods

We obtained 50 PBCs, resulting from the inoculation of blood culture (BC) bottles with GN isolates selected to represent highly diversified resistance profiles (one per bottle). Aliquots from each PBC bottle were used directly to perform the VITEK® REVEAL™ (GN01) AST assay and were plated on solid media, and overnight-grown isolates were used for broth microdilution-based AST reference assay (Figure 1). Minimum inhibitory concentration (MIC) values were interpreted using the EUCAST 2023 clinical breakpoints of antibiotics, and discrepancies were calculated according to ISO-criteria 20776/2:2007.

Results

We tested 683 combinations of bacterial organisms and antibiotics. As shown in Table 1 and, only for new beta-lactam-beta-lactamase inhibitor combinations, in Figure 2, rates of essential agreement (EA) and categorical agreement (CA) of the VITEK® REVEAL™ with the reference were, respectively, 92.6% and 94.0% for Enterobacterales organisms (n=33), 92.4% and 95.4% for Pseudomonas aeruginosa organisms (n=11), and 100% and 97.2% for Acinetobacter baumannii organisms (n=6). Very major discrepancies (VMDs) were observed for ceftolozane-azobactam (n=3), ertapenem (n=3), gentamycin (n=2), meropenem (n=1), meropenem-vaborbactam (n=1) and piperacillin-tazobactam (n=5). Major discrepancies were observed for amikacin (n=1), ertapenem (n=2) and trimethoprim-sulfamethoxazole (n=3). Five discrepancy results, which regarded antibiotics with no defined susceptible-increased exposure category, were in EA. Repeat testing led to resolve 2 VMDs (1

Enterobacterales/ceftolozane-tazobactam; 1 P. aeruginosa/meropenem). A workflow analysis revealed a mean (±SD) time to result (calculated from the time a BC flagged positive) of 6.39 (±0.08) hours for the VITEK® REVEAL™ and 40.18 (±0.11) hours for the reference assay.

Discussion and Conclusions

The VITEK® REVEAL™ system is an excellent method for rapid AST of simulated PBCs for antimicrobial-resistant bacterial pathogens. Future studies with clinical GN PBCs will be performed to confirm our findings.

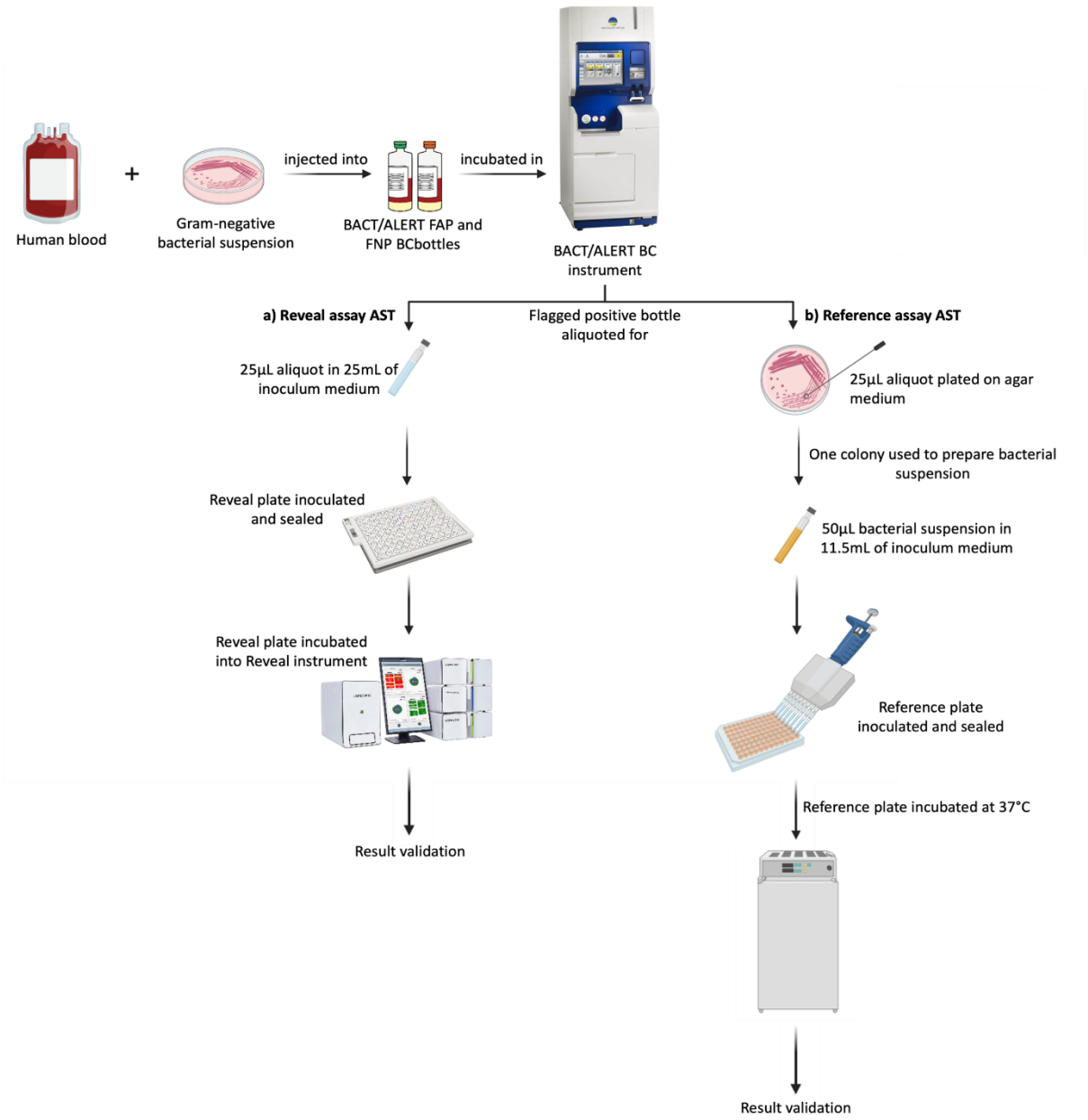

Figure 1. Antimicrobial susceptibility testing workflow

GN organisms tested against the indicated antibiotics	% of GN organisms categorised using BMD as:			% EA (n results/n tested)	% CA (n results/n tested)	% MD (n results/n tested)	% VMD (n results/n tested)	% mD (n results/n tested)
	R (n)	I (n)	S (n)					
***Enterobacterales* (n = 33)**								
• Amikacin	11	-	22	100 (33/33)	100 (33/33)	0 (0/22)	0 (0/11)	-
• Amoxicillin/Clavulanic acid	28	-	3	96.77 (30/31)	100 (31/31)	0 (0/3)	0 (0/28)	-
• Aztreonam	21	2	9	93.75 (30/32)	93.75 (30/32)	0 (0/9)	0 (0/21)	6.25 (2/32)
• Cefepime	18	6	7	96.77 (30/31)	96.77 (30/31)	0 (0/7)	0 (0/18)	3.23 (1/31)
• Ceftazidime	28	2	3	84.84 (28/33)	87.88 (29/33)	0 (0/3)	0 (0/28)	12.12 (4/33)
• Ceftazidime-avibactam	8	-	25	100 (33/33)	100 (33/33)	0 (0/25)	0 (0/8)	-
• Cefotaxime	27	0	6	96.97 (32/33)	96.97 (32/33)	0 (0/6)	0 (0/27)	3.03 (1/33)
• Ceftolozane-tazobactam	21	-	11	87.50 (28/32)	93.75 (30/32)	0 (0/11)	9.52 (2/21)	-
• Ciprofloxacin	24	0	5	100 (29/29)	100 (29/29)	0 (0/5)	0 (0/24)	0 (0/29)
• Ertapenem	15	-	18	87.87 (29/33)	84.85 (28/33)	11.11 (2/18)	20 (3/15)	-
• Gentamicin	14	-	19	93.94 (31/33)	96.97 (32/33)	0 (0/19)	7.14 (1/14)	-
• Levofloxacin	22	1	10	100 (33/33)	100 (33/33)	0 (0/10)	0 (0/22)	0 (0/33)
• Imipenem	10	0	22	100 (32/32)	100 (32/32)	0 (0/22)	0 (0/10)	0 (0/32)
• Meropenem	9	1	23	84.85 (28/33)	87.88 (29/33)	0 (0/23)	0 (0/9)	12.12 (4/33)
• Meropenem-vaborbactam	6	-	27	100 (33/33)	100 (33/33)	0 (0/27)	0 (0/6)	-
• Piperacillin-tazobactam	23	-	9	84.38 (27/32)	93.75 (30/32)	0 (0/9)	8.70 (2/23)	-
• Tobramycin	21	0	12	100 (33/33)	100 (33/33)	0 (0/12)	0 (0/21)	0 (0/33)
• Trimethoprim-sulfamethoxazole	18	1	14	87.88 (29/33)	87.88 (29/33)	7.14 (1/14)	0 (0/18)	9.09 (3/33)
• Total antibiotics	324	13	245	94.16 (548/582)	95.53 (553/582)	1.22 (3/245)	2.47 (8/324)	4.66 (15/322)
***Pseudomonas aeruginosa* (n = 11)**								
• Amikacin	2	-	9	100 (11/11)	100 (11/11)	0 (0/9)	0 (0/2)	-
• Aztreonam	1	10	-	100 (11/11)	100 (11/11)	0 (0/10)	0 (0/1)	-
• Cefepime	8	3	-	100 (11/11)	100 (11/11)	0 (0/3)	0 (0/8)	-
• Ceftazidime	7	4	-	100 (11/11)	100 (11/11)	0 (0/4)	0 (0/7)	-
• Ceftazidime-avibactam	6	-	5	90.91 (10/11)	100 (11/11)	0 (0/5)	0 (0/6)	-
• Ceftolozane-tazobactam	6	-	5	100 (11/11)	100 (11/11)	0 (0/5)	0 (0/6)	-
• Ciprofloxacin	9	1	-	100 (10/10)	100 (10/10)	0 (0/1)	0 (0/9)	-
• Levofloxacin[a]	9	2	-	100 (11/11)	100 (11/11)	0 (0/2)	0 (0/9)	-
• Imipenem	11	0	-	100 (11/11)	100 (11/11)	-	0 (0/11)	-
• Meropenem	8	3	0	100 (11/11)	100 (11/11)	0 (0/3)	0 (0/8)	0 (0/11)
• Meropenem-vaborbactam	9	-	2	90.91 (10/11)	100 (11/11)	0 (0/2)	0 (0/9)	-
• Piperacillin-tazobactam	6	5	-	90.91 (10/11)	81.82 (9/11)	0 (0/5)	33.33 (2/6)	-
• Tobramycin	6	-	5	100 (11/11)	100 (11/11)	0 (0/5)	0 (0/6)	-
• Total antibiotics	88	28	26	97.89 (139/142)	98.59 (140/142)	0 (0/54)	2.27 (2/88)	0 (0/11)
***Acinetobacter baumannii complex* (n = 6)**								
• Amikacin	3	-	3	100 (6/6)	100 (6/6)	0 (0/3)	0 (0/3)	-
• Ciprofloxacin	6	0	-	100 (6/6)	100 (6/6)	-	0 (0/6)	-
• Gentamicin	3	-	3	100 (6/6)	100 (6/6)	0 (0/3)	0 (0/3)	-
• Levofloxacin	6	0	0	100 (6/6)	100 (6/6)	-	0 (0/6)	0 (0/6)
• Imipenem	6	0	0	100 (6/6)	100 (6/6)	-	0 (0/6)	0 (0/6)
• Meropenem	6	0	0	100 (6/6)	100 (6/6)	-	0 (0/6)	0 (0/6)
• Trimethoprim-sulfamethoxazole	5	0	1	100(6/6)	83.33 (5/6)	0 (0/1)	0 (0/5)	16.67 (1/6)
• Tobramycin	4	0	2	100 (6/6)	100 (6/6)	0 (0/2)	0 (0/4)	0 (0/6)
• Total antibiotics	39	0	9	100 (48/48)	97.92 (47/48)	0 (0/9)	0 (0/39)	3.33 (1/30)
All organisms (n = 50)	**451**	**41**	**280**	**95.21 (735/772)**	**96.24 (743/772)**	**0.97 (3/308)**	**2.22 (10/451)**	**4.41 (16/363)**

Table 1. Performance of the REVEAL AST assay for 683 bacterial organism/antibiotic combinations test

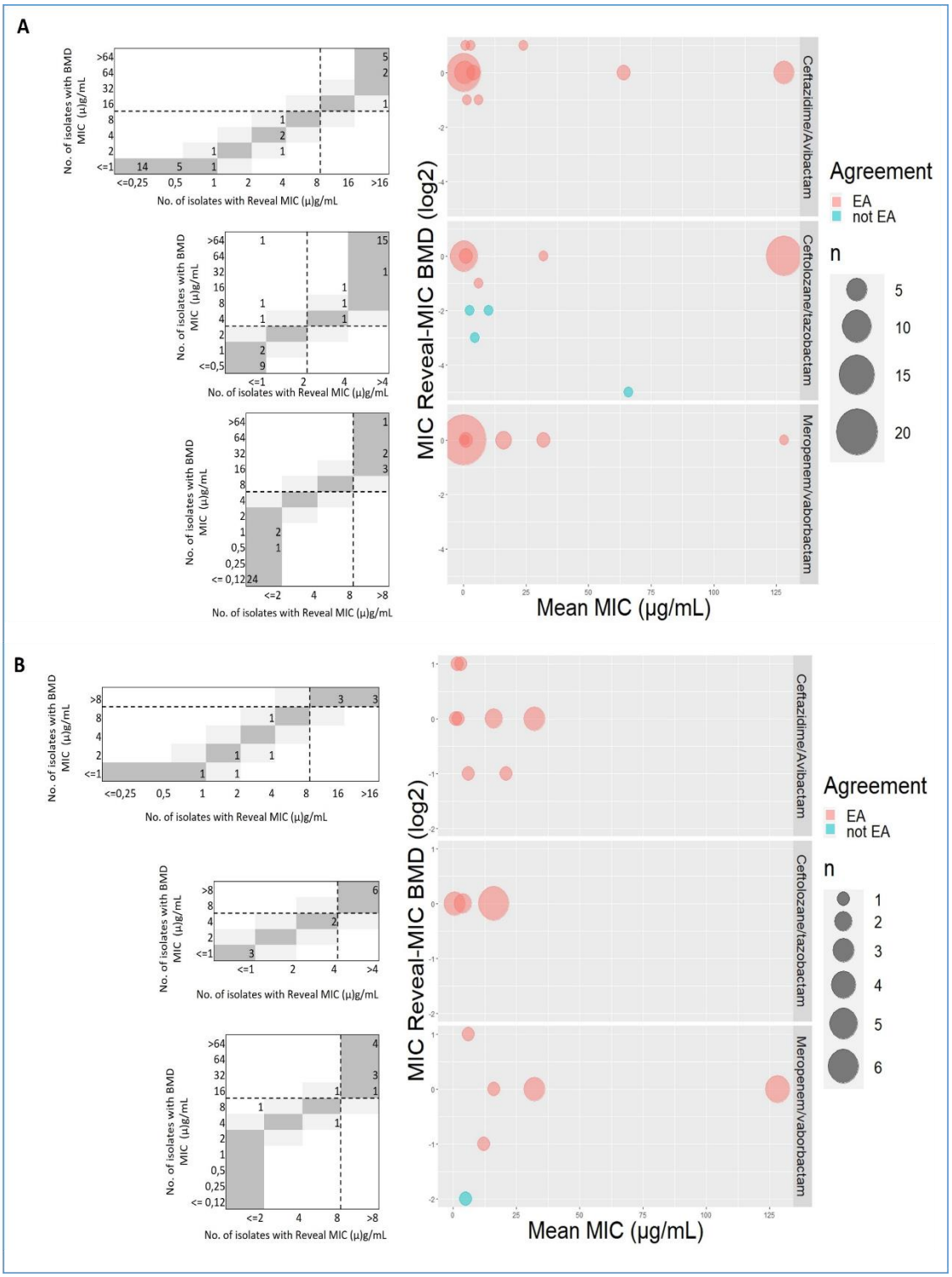

Figure 2. Performance of the REVEAL AST assay

187 - HOW DO ARTIFICIAL INTELLIGENCE-BASED SYSTEMS PERFORM IN DETECTING HEALTHCARE-ASSOCIATED INFECTIONS? A SYSTEMATIC REVIEW

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How do Artificial Intelligence-based systems perform in detecting Healthcare-Associated Infections? A systematic review

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Introduction

The use of artificial intelligence (AI)-based systems has the potential to transform the detection and diagnosis of infections. This field is particularly relevant to hospitals, which are faced with the growing complexity of managing and monitoring Healthcare-Associated Infections (HAIs) with increasing amounts of data in reduced timeframes. Despite the promise of improving the efficiency and accuracy of HAI-related traditional diagnostic procedures, the actual effectiveness of AI systems remains uncertain. The aim of this study, which updates a previous review from 2020, is to determine the performance of AI-based tools for the surveillance, detection, and control of HAIs.

Materials and Methods

The study protocol has been registered in PROSPERO (ID: CRD42024513145). A systematic review was conducted following PRISMA 2020 guidelines, accessing the following databases: PubMed, Embase, Scopus and Web of Science. Experimental and observational studies published in English, describing the use of one or more AI-based systems for detection and prediction of HAIs, were included. Outcomes of interest included the performance measures of evaluated methods, expressed as: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the receiver operating characteristic curve (AUROC), accuracy, precision, F1 score, and other performance measures.

Results

From 8,701 articles initially identified, 4,212 records were removed due to duplication. Out of 4,489 papers screened for title and abstract, 488 were included. Screening is ongoing. Preliminary results show that the HAIs which are most frequently targeted are: Urinary Tract Infections, Surgical Site Infections, Sepsis, Ventilator-Associated Pneumonia, Clostridium Difficile infections. Furthermore, we observed the use of different AI-systems, for instance Artificial Neural Network, Deep Neural Network, Natural Language Processing. Overall, significant heterogeneity was found both in the infections targeted and in types of technology. Similar results were observed between the studies in terms of healthcare settings and data sources.

Discussion and Conclusions

We observed an increase in the total number of publications compared to the previous systematic review, reflecting the growing interest in the application of AI to HAI detection. The observed heterogeneity in study designs, targeted infections, healthcare settings and data sources highlights the need for standardised methodologies and validation processes to ensure comparable results across studies, in order to maximise the real-world impact of AI tools in HAI surveillance and control efforts. Overall, the use of AI-based systems shows promising performance in HAI surveillance, early diagnosis and prediction.

188 - PERFORMANCE OF 16S RRNA GENE NEXT-GENERATION SEQUENCING AS DIAGNOSTIC TOOL IN MICROBIOLOGY SETTING

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Performance of 16S rRNA gene next-generation sequencing as diagnostic tool in microbiology setting

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Introduction. Effective treatment of infectious diseases requires prompt and accurate bacterial identification and tailored antimicrobial treatments to avoid unnecessary use or misuse of antibiotics. Traditional culture methods serve as gold standards, but their effectiveness diminishes for fastidious and hard-to-grow microorganisms. The rapid development of next-generation sequencing (NGS) technology has accelerated the clinical application of NGS in clinical microbiology laboratories as a front-line diagnostic tool. Material and methods. We analyzed data from 123 different biological samples submitted at the Department of Advanced Translational Microbiology of Trieste, Italy, for both culture-based and NGS identification between July 2022 and July 2023. The bacterial conventional method was used for isolation and species identification was performed using MALDI-TOF Mass Spectrometry. NGS identification consisted of the 16S rRNA gene (V3 region) sequencing applying the Ion Torrent PGM technology and using the Quantitative Insights into Microbial Ecology (QIIME) software for sequence data processing. Results. Among the 123 samples, drainage fluids (38%) and blood (23%) were the most common, with requests predominantly from Infectious Diseases (31.7%) and Orthopaedic Units (21.13%). In samples collected from patients with confirmed infections, NGS demonstrated diagnostic utility in over 60% of cases, either by confirming culture results in 21% or providing enhanced detection in 40% of instances. Among the 71 patients who had received antibiotic therapies before sampling (mean 2.3 prior antibiotic days), pre-sampling antibiotic consumption did not significantly affect the sensitivity of 16S NGS. Discussion and Conclusions. In routine microbiology laboratories, combining NGS with the culture method enhances the sensitivity of microbiological diagnostics, especially when sampling is conducted during antibiotic therapy.

209 - COMPARISON STUDY FOR THE DETECTION OF STIS WITH BOSPHORE CT/NG/MG PANEL KIT V1, (ANATOLIA GENEWORKS)

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Unit Of Microbiology, The Greater Romagna Area Hub Laboratory, Cesena, Italia (1) - University Of Bologna, Department Of Medical And Surgical Sciences (dimec), Bologna, Italia (2)

Comparison study for the detection of STIs with Bosphore CT/NG/MG Panel Kit v1, (Anatolia Geneworks)

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Introduction

The purpose of the study was to demonstrate the performance evaluation of the in vitro diagnostic medical device Bosphore CT/NG/MG Panel Kit v1 from Anatolia Geneworks. The kit is intended for the qualitative detection of specific DNA regions of Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG) and Mycoplasma genitalium (MG), using the Real-Time PCR method. CT and NG are gram-negative bacteria, while MG is a gram-positive bacterium, and all of them can cause sexually transmitted infections. Symptoms for CT and NG infections may include discharge, painful urination, and pelvic pain, while MG infections may cause urethritis or cervicitis. The diagnosis and treatment of sexually transmitted infections is a crucial component of providing evidence-based care.

Materials and methods

A total of 230 samples from different matrices (Table 1), were retrospectively collected at the Unit of Microbiology of the Greater Romagna Hub Laboratory (Emilia Romagna Region, Italy). Bosphore CT/NG/MG Panel Kit v1 is an in vitro diagnostic Real-Time PCR kit intended for qualitative detection of C. trachomatis, N. gonorrhoeae and M. genitalium bacterial DNA extracted from vaginal swab, endocervical swab, urethral swab, urine, eye swab. DNA was extracted with UNIO Bacterial DNA Extraction Kit on UNIO B24 Extraction System from Anatolia Geneworks. The real-time PCR was performed by Montania 4896 Real-Time PCR instrument, using the Bosphore CT/NG/MG Panel Kit v1. Results were compared to those obtained from reference devices used for diagnostic routine.

	Sample results	
Sample type	Positive sample	Negative sample
Vaginal swab	20	25
Endocervical swab	20	25

Urethral swab	20	25
Eye swab	20	25
Urine	25	25

Table 1. Number and type of samples tested.

Results

The results met the requirements of MDCG 2022-2 - Guidance on general principles of clinical evidence for In Vitro Diagnostic medical devices (IVDs). The statistical results are within the acceptable range in the clinical performance evaluation, and it was observed a complete concordance between the two diagnostic methods. Bosphore CT/NG/MG Panel Kit v1 from Anatolia Geneworks is an eligible test that complies with WHO and ECDC.

Discussion and Conclusions

Each year, there are an estimated 374 million new infections with one of three curable STIs: chlamydia, gonorrhea, and trichomoniasis. Given the spread of these infections, which are often asymptomatic, it is essential to diagnose them effectively. The overall conclusion is that Bosphore CT/NG/MG Panel Kit v1 met the acceptance requirements, and the sensitivity and sensibility were in line with the intended use. Therefore, Bosphore CT/NG/MG Panel Kit v1 from Anatolia Geneworks is a promising method in STIs diagnostic field.

220 - NASOPHARYNGEAL AND SALIVA SAMPLES EVALUATION FOR SARS-COV-2 INFECTION DIAGNOSIS IN PEDIATRIC POPULATION.

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Nasopharyngeal and saliva samples evaluation for SARS-CoV-2 infection diagnosis in pediatric population.

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Introduction: The diagnosis of respiratory infections is based on nasopharyngeal sampling. Nasopharyngeal swabbing is unconfortable in patients. In particular, children were often not collaborative, and the sample could not be correctly performed, leading to false negative results. In addition, this collection procedure requires trained healthcare personnel and increases the risk of aerosol exposure for healthcare workers. This study compares nasopharyngeal swab and saliva swab samples in the molecular diagnosis of SARS-CoV-2 infection in order to evaluate a possible alternative in sampling for respiratory disease diagnosis.

Material and methods: We randomly enrolled 256 pediatric patients in the first SARS-CoV-2 pandemic era (from September 2020 to December 2020). All patients were admitted to the pediatric Emergency Department of AOUI, Verona. We collected paired nasopharyngeal and saliva swab samples from each patient. Nucleic acids were extracted from samples using the Nimbus system (Nimbus, Seegene) and amplified using a COVID-19 kit (Seegene) using multiplex RT-PCR procedure, detecting E, RdRp/S, and viral N genes. We considered the sample positive when at least a single positive viral gene target showed a cut-off cycle threshold value of 40. We statistically analyzed our data with MedCal Software and using a Mann-Whitney U-test.

Results: We analyzed this cohort by dividing little patients into two groups: Group A with patients younger than 6 years old (115 boys and 75 girls) with a median age of 1.92 years and Group B with

patients older than 6 years (36 boys and 30 girls) with a median age of 10.92 years. SARS-CoV-2 genes were detected in the nasopharyngeal swab samples of 16 out of 256 patients, whereas 13 out of 256 were positive in the saliva swab samples. Overall, concordance between nasopharyngeal and saliva swab samples was detected in 253 out of 256 samples (98.83%). The sensitivity was 81.25%, and the specificity was 100%. Our samples showed little difference in the Ct value average detected in nasopharyngeal and salivary swab-positive samples. In particular, the Ct value of salivary samples was slightly higher than that of nasopharyngeal ones, but these differences were not statistically significant.

Conclusion: Our results suggested that the collection of saliva swab samples in children could be a good alternative to nasopharyngeal swab in particular for children in pre-school age. This sampling method could help in improving diagnosis and tracing of children with SARS-CoV-2 infection.

221 - THE INTRODUCTION OF THE BCID2 BIOFIRE PANEL IN THE DIAGNOSTIC FLOW-CHART IN THE LABORATORY OF THE "F. SPAZIANI" HOSPITAL, FROSINONE

Federica Maria Di Lella [(1)] ***- Camilla Bitossi*** [(1)] ***- Catia Sias*** [(1)] ***- Amelia Terrinoni*** [(1)] ***- Rossana Pulselli*** [(1)] ***- Alessandro Bracaglia*** [(1)] ***- Maria Calzetta*** [(1)] ***- Daniela Fanfarillo*** [(1)] ***- Graziella Frabotta*** [(1)] ***- Adriana Letta*** [(1)] ***- Giacinto Panella*** [(1)] ***- Carla Gargiulo*** [(1)]

Fabrizio Spaziani, Ospedale Di Frosinone, Frosinone, Italia [(1)]

The introduction of the BCID2 BioFire panel in the diagnostic flow-chart in the laboratory of the "F. Spaziani" Hospital, Frosinone

FEDERICA M. DI LELLA, CAMILLA BITOSSI, CATIA SIAS, LIVIA DE STEFANO, AMELIA TERRINONI, ROSSANA PULSELLI, ALESSANDRO BRACAGLIA, MARIA CALZETTA, DANIELA FANFARILLO, GRAZIELLA FRABOTTA, ADRIANA LETTA,GIACINTO PANELLA, CARLA GARGIULO

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Introduction

Bacteremia and sepsis are devastating diseases associated with high mortality and sequelae. Therefore, timely administration of target therapy is fundamentalin improving a patient's outcome. It is hence really useful to have fast methods in the laboratory that allow us to speed up the identification of the etiological agent. The FilmArray blood culture identification (BCID) is a molecular test for the direct identification of BSI-causing pathogens from positive blood cultures (BCs) and consents the detection of the most important resistance genes in a short time.

Materialsand Methods

This study was conducted at the laboratory of the Frosinone Hospital, a multifunctional hub that also accepts samples from neighboring hospitals included in the ASL of Frosinone, for a total of 542 beds. BC bottles (aerobic and anaerobic BacT/Alert) were incubated in BACT/ALERT® VIRTUO® (bioMérieux) at 37°C. The BCs considered for BCID2 analysis were those that became positive in less than 24 hours (TP), belonging to critically ill patients and newborns. FilmArray BCID2 testing was performed according to the manufacturer's guidelines. In parallel, positive bottles were plated on specific culture media and incubated at 37°C. The bacteria obtained from the cultures were subjected to identification using MALDITOF (Vitek MS prime bioMérieux) and to susceptibility testing on VITEK® 2 AST (bioMérieux) using the relevant card.

Results

Between 1 September 2023 and 31 December 2023, 81 BCID2 tests were performed. Of these, 3 monomicrobial runs revealed an off-panel organism and 2 run was called invalid. Therefore, these have not been included in the performance analysis. Here, the clinical performance of the BCID2 assay for species identification in 76 positive BCs was evaluated. BCID2 results were concordant with the gold standard (culture and phenotypic susceptibility testing) in 55/76 (72.4%) BCs. Nonconcordance was related to detection of additional pathogens by the BCID2 assay (n = 4), discrepant species identification (n = 12), or failure of BCID2 to detect pathogens on the panel (n = 1), polymicrobial BCs (n = 4). BCID2 identified the presence of bla-KPC-carrying species in 6 and blaCTX-M in 3 BCs. Methicillin resistance was successfully detected in 11 BCs.

Discussion and Conclusions

The introduction of "Fast Microbiology" can improve patient outcomes, reduce mortality and save the use of broad-spectrum antibiotics. The BCID2 showed good overall concordance with conventional species identification, principally in monomicrobial cultures. Despite several limitations, in conclusion, BCID2 proves to be a reliable and useful test for the rapid identification of microorganisms causing BSI from positive blood cultures.

228 - IMPACT OF REAL-TIME PCR ON WHIPPLE'S DISEASE DIAGNOSIS: THE EXPERIENCE OF FONDAZIONE IRCCS POLICLINICO SAN MATTEO

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Title: Impact of real-time PCR on Whipple's disease diagnosis: the experience of Fondazione IRCCS Policlinico San Matteo

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Introduction: Tropheryma whipplei is a rod-shaped actinomycete extensively present in the environment. T. whipplei is responsible for Whipple's disease (WD), a rare systemic infection that primarily affects the small intestine. Symptoms are non-specific, long-term and include arthralgia, fever, weight loss, diarrhea, abdominal pain, joint pain, and neurological complications.

Many patients may receive a later diagnosis of WD due to non-specific symptoms and low incidence of the disease. Small bowel biopsy and an upper tract endoscopy is recommended when WD is suspected. Indeed, the gold standard for WD diagnosis is the histological detection of foamy macrophages containing large amounts of diastase-resistant Periodic Acid Schiff (PAS)-positive particles in the lamina propria of the duodenum. PCR testing should be employed when PAS staining is negative, but WD is clinically suspected. We describe our experience using real-time PCR assay for the detection of T. whipplei, introduced at Fondazione IRCCS Policlinico San Matteo in January 2021.

Materials and methods: The real time PCR reaction targeted the groEL gene of the bacterium (BactoReal® Kit Tw-Ingenetix). From January 2021 to May 2024 139 samples including small bowel biopsies, cerebrospinal fluid (CSF), synovial fluid, urine, stool, and saliva were tested. The 139 samples were collected from 80 patients with clinical suspicion of WD.

Results: T. whipplei presence was detected in fourteen samples from ten out of 80 patients (12,5%). Seven/10 were male, median age was 57 (range: 40-76 years-old). Eight/10 patients underwent duodenal biopsy, and for one of them stool and saliva were also positive. The remaining two positive samples were from CSF and synovial fluid. Clinical conditions and anatomic pathology response confirmed WD for 8 out of 10 patients.

Discussion and conclusions: The non-specific symptoms, the presence of carriers, and a negative PAS staining in almost half of the cases, make WD diagnosis still difficult. T. whipplei detection via PCR in duodenal biopsies has been proved in our experience as a reliable and valid support in diagnosis. PCR detection of T. whipplei from other clinical specimens such as stool, urine, saliva must be contextualized with the clinical status to avoid false positives due to colonization.

241 - METAGENOMICS APPROACH FOR VIRAL PNEUMONIA DIAGNOSIS

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Metagenomics approach for viral pneumonia diagnosis

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Introduction

Lung infections are the most common and important infectious diseases due to the high morbidity and mortality, especially in elderly and immunocompromised individuals. However, due to the sensitivity limitations and long Turnaround Time of conventional diagnostic tests, pathogen detection and identification methods with higher diagnostic efficiency are urgently needed. In recent years, next-generation metagenomic sequencing (mNGS) has been widely implemented to detect rare and emerging pathogens, showing, in some aspects, a more efficient diagnostic performance than conventional methods. In this context, the aims of this study were to:

(i) Develop and validate a mNGS protocol for the sequencing of clinical specimens collected from human patients with clinical respiratory syndromes.

(ii) Compare the performance of the mNGS protocol against standard virological diagnostic methods.

Material and Methods

A total of 197 respiratory samples (nasopharyngeal swab and bronchoalveolar lavage) collected from patients with influenza-like illness or acute respiratory infection were tested with a multiplex real-time RT-PCR panel for respiratory viruses, or with syndromic respiratory panels. mNGS sequencing will be performed in both samples in which one or more viruses could be identified as well as in those in which a pathogen could not be identified. The sequences obtained were analyzed with bioinformatics tools or with an in-house pipeline.

Results

To date, the mNGS protocol has been performed on 42 /197 respiratory samples (21.3% of the total). The sequences obtained and analyzed were compared with the results of routine diagnostic tests to

investigate the concordance between the two techniques. From our preliminary data, 32/42 samples (76.2%) showed concordance between mNGS results and diagnostic results (24 positive and 8 negative). 10/42 samples (23.8%) showed discordance between the 2 approaches: in 9 samples positive for a single virus with the diagnostic tests, coinfections were detected thanks to mNGS; while in 1 sample with no identified clinical diagnosis, mNGS identified Rhinovirus E15.

Discussion and Conclusions

So far, mNGS cannot replace conventional pathogen detection methods, but its unbiased pathogen detection capabilities and the ability to collect epidemiological information helps to increase the diagnostic yield. In this regard, whenever high values of genomic coverage and depth of sequencing are obtained using mNGS - in addition to viral identification – it can be possible to conduct genetic and phylogenetic analyses to gather more epidemiological information. In particular, it may prove to be an excellent approach strategy when mixed or rare pathogen infections are suspected; especially in immunocompromised individuals or those with serious conditions requiring urgent treatment.

242 - PARALLELING EPIGENETIC PROFILES AND EARLY-LIFE MICROBIAL COLONIZATION IN ASD PATIENTS AND IN A MOUSE MODEL OF BACTERIAL DEPLETION: SEARCHING EARLY BIOLOGICAL MARKERS FOR NEURODEVELOPMENTAL DISEASES.

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Paralleling epigenetic profiles and early-life microbial colonization in ASD patients and in a mouse model of bacterial depletion: searching early biological markers for neurodevelopmental diseases.

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1. Introduction: Early perturbation of the microbiota-gut-brain axis can influence neurological outcomes, altering behavior and affecting the onset and severity of Autism Spectrum Disorders (ASD). A few studies have focused on the correlation between gut dysbiosis and epigenetic modification in the host, even though epigenetic processes are prominent in the regulation of early cellular differentiation and in defining stable changes in cellular phenotype. We defined the methylation signatures of human host fecal DNA (HFD) from an age-restricted cohort of young children with ASD for whom we previously determined fecal microbiota (FM) composition. More recently, a mouse model of early-life antibiotic-induced bacterial depletion has been also employed to define DNA methylation profiles in intestine and brain cells and behavioral outcomes. 2. Materials and methods: DNA methylation analyses of HFD, colon mucosa and brain areas were performed using the Infinium MethylationEPIC array followed by Gene Ontology Enrichment analysis of differentially methylated genes and promoters and cell composition analysis. 3. Results: along with marked gut dysbiosis, specific epigenetic signatures in HFD at genes related to inflammation associated with the disease. Methylation-based deconvolution algorithm revealed that the HFD derived mainly from immune cells. Along with metataxonomic analysis of FM, results showed a clear acceleration of epigenetic age in ASD children that correlates with the alteration in abundance of specific colonizers marking the transition from an infant- to adult-like gut microbiota. Interestingly, the use of a preclinical mouse model of early-life antibiotic exposure showed a re-shaping of DNA methylation profiles in the colon mucosa and at specific brain areas. 4. Discussion and conclusions: This study aimed to gather new insights into the paradigm of the microbiota-gut-brain axis, namely in the role of gut and brain epigenetics in integrating gut microbiota perturbations in early life. Furthermore, appropriate

enlargement and extension of the study to both animal models and patients could be important to causally link the onset of gut dysbiosis to methylation patterns responsible for cell programming and functions and to improve the identification of early biological markers for neurodevelopmental human diseases.

259 - THE EXPRESSION OF CD169 AND HLA-DR IN BLOOD CELLS AND CIRCULATING MICROVESICLES AS A NEW TOOL FOR MONITORING SARS-COV-2 INFECTION AND LONG COVID ASSOCIATED SEQUELAE: LESSON FROM PANDEMIC WAVES

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The expression of CD169 and HLA-DR in blood cells and circulating microvesicles as a new tool for monitoring SARS-CoV-2 infection and Long COVID associated sequelae: lesson from pandemic waves

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Introduction: Elevated inflammation and immune dysregulation are main consequences of SARS-CoV-2 infection. The dysregulated inflammatory state persists after COVID-19, generating the post-acute sequelae of SARS-CoV-2 infection in Long COVID individuals (LC). The role of CD169 as a marker in the early diagnosis of SARS-CoV-2 infection and its association with severity and clinical outcome was demonstrated in COVID-19 patients (COV) in the period between 2020 and 2021. On these bases, we evaluated the expression of CD169 and HLA-DR on monocytes and on circulating microvesicles (MVs) from COV and LC to better elucidate their involvement in immunological dysfunction and in association with serum inflammatory markers and clinical features. The possible impact of different pandemic waves was also evaluated. Materials and Methods: Blood samples from 133 COV, 132 LC (7-48 weeks post-infection), and 59 Healthy Donors (HDs) were collected at Tor Vergata University Hospital of Rome. COV and LC were characterised according with severity, pneumonia involvement,

respiratory outcome and treatments during acute phase of SARS-CoV-2 infection, and comorbidity aspects.

Leukocytes were characterized for HLA-DR and CD169 and, in a subset of COV and LC, were also analyzed their expression on MVs. Serum inflammatory markers were assessed by Ella immunoassay system. Flow cytometry for immunophenotyping and MVs characterization was performed by CytoFLEX. Results: CD169 RMFI was found significantly higher in COV than in HDs and LC, resulting as good marker of viral infection. Whereas the percentage of CD169+ monocytes was high in COV and LC, and the percentage of HLA-DR+ monocytes was low whit respect to HDs. Notably, the percentage of activated monocytes CD169+HLA-DR+ were high in COV and persisted in LC. the alterations of CD169 and HLA-DR expression and indices of inflammation were also observed upon different COVID-19 waves. The percentage of HLA-DR+CD169+ cells correlated with inflammatory markers in COV and LC. In plasma, the percentage and number of HLA-DR+CD169+ MVs were significantly elevated in COV and, although lower, persist in LC compared to HDs. Discussion and conclusions: CD169 RMFI and myeloid activation markers were confirmed as predictive markers of COVID-19, and myeloid activation persisted in LC. A dynamic correlation among CD169 and HLA-DR expression was found at cellular level and MVs in association with inflammatory cytokines and coagulation factors, drawing attention to MVs phenotyping for monitoring emerging respiratory viruses associated diseases.

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268 - EVALUATION OF THE SYNDROMIC MENINGO-ENCEPHALITIS TEST FOR THE VIRAL PATHOGENS IDENTIFICATION IN PATIENTS WITH ACUTE NEUROLOGICAL SYNDROME

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Evaluation of the syndromic meningo-encephalitis test for the viral pathogens identification in patients with acute neurological syndrome

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Introduction

Acute infection of the central nervous system (CNS) is a severe disease and a rapid identification of the specific etiology is essential for providing a prompt therapy which is crucial to minimize neurological permanent damages and death. Several studies highlighted the usefulness of syndromic tests for a rapid diagnosis, despite limited data exist evaluating the implementation of those multiplex assays testing for meningitis or encephalitis. In the present study we evaluated the analytical performance in the viral etiology diagnosis of the BioFire, FilmArray Meningitis/Encephalitis (FA-ME) syndromic panel, by comparing these results with those obtained with virus specific realtime-PCR/RT-PCR (PCRs).

Materials and Methods

Cerebrospinal fluid (CSF) from 4859 patients with acute meningitis or encephalitis was analyzed by using the FA-ME test, from August 2016 to February 2024. The test simultaneously detects a panel of 6 bacteria (S. pneumoniae, N. meningitidis, S. agalactiae, E. coli K1, L. monocytogenes, H. influenzae), 7 viruses (HSV-1, HSV-2, VZV, Enterovirus (EV), Human Parechovirus (HPeV), HHV6, CMV) and 2 fungi (Cryptococcus gatii/neoformans). A total of 828/4859 CSFs underwent further testing with virus specific PCRs for further clinical investigation. Then, the diagnostic accuracy of the FA-ME test was compared with the individual PCRs, using a weighted 2 x 2 kappa coefficients analysis, for the following viruses (No. samples): HSV-1 (188), HSV-2 (190), VZV (159), CMV (124), HHV6 (36) and Enterovirus (101), HPeV (30).

Results

Pathogens were identified in 681/4859 CSFs [EV (98), HPeV (15), HSV1 (78), HSV2 (19), VZV (99), HHV6 (66), CMV (15), S. pneumoniae (148), N. meningitidis (32), L. monocytogenes (39), H. influenzae (28), E. coli K1 (10), S. agalactiae (7), Cryptococcus neoformans/gattii (12) and co-infected (15)]. Results showed that viral pathogens were most commonly detected (57,27%), followed by bacteria (38.8%), and fungi (1.8%). The Kappa coefficient analysis revealed an almost perfect agreement between the two methods for 5/7 viruses (κ>0.8): VZV (κ=1.0), HSV-1 (κ=0.92), HSV-2 (κ=1.0), HHV-6 (κ=0.83) and EV (κ=0.83); a substantial agreement was also obtained for CMV (κ=0.79). Instead, for HPeV, since no positive samples were available, it was not possible to calculate the agreement between the two tests.

Discussion and Conclusions

Our data show a high diagnostic accuracy of FA-ME test compared to virus specific realtime PCRs, considered the gold-standard for meningo-encephalitis diagnosis. Particularly, the syndromic assay showed high agreement with PCRs for VZV, HSV-1, HSV-2, HHV-6 and EV, and a substantial agreement for CMV. Further studies are needed to evaluate the diagnosis of HPeV using FA-ME test.

271 - PARVOVIRUS B19 INFECTION IN A WOMAN AFFECTED BY PSORIASIS, A CASE REPORT.

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Parvovirus B19 infection in a woman affected of psoriasis, a case report.

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Background

Parvovirus B19 is a small DNA virus, member of the genus Erythrovirus of the family Parvoviridae. Most infections in healthy adults are either asymptomatic or characterized by mild symptoms such as transient fever, malaise, myalgia and headache. Psoriasis is a common chronic, immune-mediated inflammatory skin disease and a variety infective pathogen can play key role in induction and exacerbation of this disorder. Many studies reported the correlation between Parvovirus B19 infection and psoriasis, but pathophysiological interactions needed future studies.

Description of case

We present a case report of a 38-year-old woman affected by psoriasis with a very high level of Parvovirus B19 detected by molecular test. The patient was admitted to the Emergency Department with fever, myalgias atralgias. skin rash all over the body accompanied by itching. She revealed a history of cutaneous psoriasis in the trunk and scalp, treated with topical drug. Haematological analyses displayed alterations in haemachrome, D-Dimer, alkaline phosphatase, albumin, positivity to EBNA and VCA Epstein Barr virus (IgG) and a very high Parvovirus B19 viral load of 3.370305 number copies/ml. All symptoms were compatible with infective status of patient. Symptomatic treatment with anti-inflammatory and antihistamine drugs performed and patient returned at home. After 25 days of first hospital access the subject had already high viral load of 19528 number copies/ml and psoriatic plaques on the neck.

Discussion and Conclusions

Several case reports have suggested associations between Parvovirus B19 infection and various chronic autoimmune and dermatologic diseases. In arthritis, protein NS1 of Parvovirus B19 is known to induce the secretion of proinflammatory cytokines. These cytokines cause inflammation and cell damage and are also found in psoriasis, as reported in literature. Considering the case examined, we believe that the search for Parvovirus B19 by a specific serology/or molecular tests in the presence of psoriasis is important, because the clinical manifestations of the infection are like the presentation of some immunological diseases are a common origin, and because Parvovirus B19 has a potential ability to trigger autoimmune diseases in genetically predisposed subjects.

274 - PULMONARY MICROBIOTA COMPARISON IN ICU VERSUS NON-ICU SETTINGS. THE MULTICENTER PULSE STUDY

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Pulmonary Microbiota Comparison in ICU versus Non-ICU Settings. The multicenter PULSE study

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INTRODUCTION. Although the lung is thought to be a sterile microenvironment in healthy subjects, recent studies showed that various bacterial species are present in the lower respiratory tract of healthy subjects, including genera such as Prevotella, Veillonella and Streptococcus. The functions of the microbiota are important for healthy subjects and include protection from pathogens and modulation of the immune response. The composition of the pulmonary microbiota is determined by the balance of three factors: migration of microorganisms into the airways, elimination of microorganisms from the airways, and the reproduction rate of the population found within the airways. The aim of this work was to study the pulmonary microbiota of patients who develop pneumonia with severe acute respiratory failure, for which Intensive Care Unit (ICU) admission is necessary, and to compare it with that of patients diagnosed with pneumonia, but who do not develop severe acute respiratory failure and subsequent ICU admission. MATERIALS AND METHODS. In this

bicentric, prospective observational study, 33 patients (more than 18 years old) were enrolled: 10 non-ICU patients, 11 ICU patients and 12 healthy cadaver controls. Samples were collected by bronchoalveolar lavage. Genomic DNA was extracted from the samples using QIAmp Microbiome kit. The preparation of 16S libraries was carried out using the Microbiota solution A kit (V3-V4). Sequencing was performed on the MiSeq platform. Sequences were analyzed using MicrobaT software and statistical analysis was performed using MicrobiomeAnalyst software. RESULTS. Among the 33 collected samples, 12 were negative for clinical detection of infection, 5 positive for fungi and bacteria, 7 for Gram negative and 4 for Gram positive. The microbiota associated with the controls was 42% Firmicutes, 17.4% Proteobacteria, 13.7% Bacteroidetes, 8.5 % Actinobacteria and 0.4% Verrucomicrobia. Comparing ICU patients with non-ICU patients, a specific signature associated with the two groups was determined. In particular, the presence of Massilia timonae associated with non-ICU patients was highlighted. No specific associations are observed when considering the different pulmonary colonization. DISCUSSION AND CONCLUSION. The phylum composition of the lung microbiota observed in this study confirms what is found in the literature. In contrast, the presence of Massilia timonae, a non-fermentative aerobic gram-negative rod, associated with subjects with a positive prognosis, is not reported in the literature. On the basis of the obtained results, it is possible to hypothesize a protective role played by this bacterium, by for example, a competition and/or inhibition phenomenon towards other colonizing species.

278 - RAPID MOLECULAR DIAGNOSTICS OF RESPIRATORY INFECTIONS IN PEDIATRIC PATIENTS: THE EXPERIENCE OF POLICLINICO UMBERTO I IN ROME

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Rapid molecular diagnostics of respiratory infections in pediatric patients: the experience of Policlinico Umberto I in Rome

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Introduction

Syndromic panels allowed laboratories to rapidly provide highly sensitive and specific test results for a broad range of viruses and bacteria causing upper respiratory illness. Aim of the present study was to describe the incidence of rapid molecular diagnostics of respiratory infection in pediatric patients at Policlinico Umberto I in Rome.

Materials and Methods

A retrospective observational single-center data study of pediatric patients with suspicion of acute respiratory infection was conducted. The rapid syndromic respiratory panel QIAstat-Dx® Respiratory SARS-CoV-2 Panel was used, an assay able to detect the following pathogens: Flu A, Flu A H1N1 pdm09, Flu A H1, FluAH3, Flu B, Coronavirus (CoV) 229E, CoV HKU1, CoV NL63, CoV OC43, SARS-CoV-2, Human parainfluenza virus1 (HPIV1), HPIV2, HPIV3, HPIV4, Respiratory syncytial virus A/B (RSV), Human Metapneumovirus A/B, Adenovirus, Bocavirus, Rhinovirus/Enterovirus, Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella Pneumoniae, Bordetella pertussis.

Results

The results of 388 respiratory samples collected during 9-months period (September 2023 to April 2024) from pediatric patients (190 female/198 male, median age [IQR]: 1.0 [0.5-4.7] years) were retrospectively analyzed. Overall, 296 (76.3%) were positive and 203/296 (68.6%) showed monoinfection while 93/296 (31.4%) coinfection. Among coinfections, 81/93 (87.1%) were positive for two pathogens, 11/93 (11.8%) for three pathogens and only 1/93 (1.1%) was positive for four pathogens.

Overall, 126 out of 296 respiratory samples (42.7%) revealed Rhinovirus/Enterovirus, 87 (29.5%) RSV, 33 (11.2%) Human Metapneumovirus A/B, 25 (8.4%) HPIV (1, 2, 3 and 4), 24 (8.1%) Bocavirus, 23

(7.8%) CoV (OC43, HKU1, NL63), 19 (6.4%) Bordetella pertussis, 18 (6.1%) Adenovirus, 18 (6.1%) Flu B, 13 (4.4%) Flu A H1N1 pdm09, 9 (3.1%) Mycoplasma pneumoniae, and 5 (1.7%) SARS-CoV-2. Conversely, among monoinfection, 63 out 203 (31.0%) respiratory samples revealed Rhinovirus/Enterovirus, 58 (28.6%) RSV, 18 (8.9%) Bocavirus, 14 (6.9%) Flu B, 13 (6.4%) Metapneumovirus A/B, 12 (5.9%) Flu A H1N1 pdm09, 9 (4.4%) Adenovirus, 8 (3.9%) CoV (OC43, HKU1, NL63), 7 (3.4%) Bordetella pertussis, 6 (3.0%) HPIV (1, 2, 3 and 4), 4 (2.0%) Mycoplasma pneumoniae, and 1 (0.5%) SARS-CoV-2.

Discussion and Conclusions

The use of syndromic panel testing has showed a high number of coinfections and the detection of some “orphan” viruses of any clear clinical impact (bocavirus and HPIV4). Our data provide information about the epidemiology of several respiratory pathogens and coinfection also suggesting that it is important to inform clinicians to facilitate interpretation because results are qualitative it is not always obvious which pathogen is responsible for a patient’s symptoms.

279 - HOW CAN A CANDIDA REAL-TIME PCR COMPLEMENT CULTURE BASED DIAGNOSTICS OF CANDIDEMIA?

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How can a Candida real-time PCR complement culture-based diagnostics of candidemia?

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INTRODUCTION: Invasive Candidiasis (IC) diagnosis is currently witnessing a growing need for nonculture-based tests to enhance sensitivity and specificity of the available culture based and biomarker testings. Given the potential role of real-time Candida PCR in satisfying this need by detecting Candida species directly from clinical samples, we aimed to:

- determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of a real-time Candida PCR and,

- design a diagnostic scheme into which Candida PCR can be incorporated into patient's care and shed some light on the benefits of combination testing.

MATERIALS AND METHODS: We performed a retrospective analysis to evaluate the performance of a commercially available OLM CandID real-time PCR (OLM Diagnostics, Newcastle-upon-Tyne, UK) in our setting. We collected surplus serum from 110 samples previously drawn for beta-D-Glucan testing (beta-D Glucan Test Wako, Wako Pure Chemical Industries) per our routine diagnostic procedures. The collected sera comprised cases of possible, probable, and proven IC following the EORTC/MSGERC criteria and based on reported blood culture (BC) and beta-D-Glucan (BDG) results.

RESULTS: A total of 40 (40/110) sera have been tested and the PCR performance results showed a 100% specificity (23/23), 64% sensitivity (11/17), 100% PPV (11/11) and 80% NPV (23/29). The only 2 initially false positive PCRs were later confirmed by BC, anticipating BC results by 3 and 5 days, respectively. False negatives were attributed to possible BC catheter contamination and pre-analytical DNA degradation given the retrospective nature of the study. On the light of those preliminary results, we have designed a diagnostic flowchart with the BDG as the primary screening test and the Candida PCR as a complementary test to BC.

DISCUSSION AND CONCLUSIONS: Our analysis yielded promising outcomes, demonstrating excellent agreement between molecular and culture identification methods. Hence, integrating Candida real-time PCR into routine practice shows potential for delivering more timely and dependable diagnoses of candidemia. Additionally, expanding our sample size and including samples from patients with probable and proven intra-abdominal candidiasis can further enrich our findings. Looking ahead, prospectively evaluating the sensitivity of our algorithm as a whole, rather than solely focusing on the PCR's sensitivity, can offer a more precise assessment of its clinical performance.

T02 FISIOLOGIA E METABOLISMO MICROBICO

11 - DESIGNING MICRO-B, A NOVEL DYNAMIC IN VITRO MODEL TO STUDY THE HUMAN GUT MICROBIOTA

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Designing MICRO-B, a novel dynamic in vitro model to study the human gut microbiota

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Introduction. Several in vitro models mimicking in vivo intestinal topography and conditions were designed and made available to the scientific community in recent years. The growing interest in such models arose from the need to circumvent the main limitations of working with animals and humans. However, the application of artificial devices in the field of the gut microbiota is still limited. In the present study, a previously designed static in vitro model was mechanically improved by implementing a constant flow and peristalsis-like movements, and gut microbiota features were evaluated over time under such conditions.

Materials and Methods. Gelatin membranes biofabricated by electrospinning were used as scaffolds for microbial adhesion and growth in the in vitro model. In vitro cultures of the gut microbiota in the newly designed model, named MICRO-B, were separately performed under static conditions (S), dynamic conditions at a medium flow rate of 1.25 ml/min (F), physiological peristalsis with 10% deformation and a frequency of 0.15 Hz (P), and combining both flow and peristalsis (F+P) for 24h, 48h, and 72h. Crystal-violet assays were performed on the scaffolds to evaluate the biofilm formation and adhesion of gut microorganisms in such conditions at different time points. Bacterial 16S rRNA gene-targeting Real-Time quantitative PCRs were performed to quantitatively assess the composition of the communities grown on the scaffolds at phylum and genus levels. Vitamins and short-chain fatty acids (SCFAs) were quantified in the culture supernatants.

Results. The mechanical setup of the in vitro model under the different conditions was stable for up to 72h. Differences in biofilm formation among the four conditions were evidenced, highlighting that biofilms in S were significantly less abundant than those in F, P, and F+P at each time point, whereas F, P, and F+P displayed similar results in terms of biofilm formation. Quantitative differences in specific phyla and genera were also pointed out. Experiments for vitamin and SCFA quantification are ongoing.

Discussion and Conclusions. The newly designed MICRO-B model resulted in a suitable platform for culturing the gut microbiota, demonstrating fluctuations in the biofilm formation, microbial composition, and metabolite secretion of the gut microbiota in response to the different imposed stimuli. Therefore, the implementation of flow and peristalsis represents a sensitive improvement of the previous static system, allowing the platform to get closer to the physiological environmental conditions found in the intestine. Further experiments will be carried out to corroborate this hypothesis.

37 - THE IMPACT OF FATTY ACID SYNTHASE ON HSV-1 INFECTION DYNAMICS

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The impact of fatty acid synthase on HSV-1 infection dynamics

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Introduction. Herpes simplex virus type-1 (HSV-1) is a widespread human pathogen that relies on host cell pathways, including those involved in metabolism to support replication. Due to its neuroinvasive and neurotoxic capacity, HSV-1 has been associated with several neurodegenerative diseases, including Alzheimer's disease (AD). This study aims to understand the intricate relationship between viral infection and cellular metabolism to develop effective interventions against HSV-1 and identify potential targets for AD prevention. Materials and Methods. SH-SY5Y neuronal-like cells were infected with HSV-1 to establish an in vitro model of HSV-1-associated neuronal pathologies. Cells were treated with fatty acid synthase (FASN) inhibitors (CMS121, C75) to evaluate their antiviral effects. Additionally, to elucidate the relationship between FASN and HSV-1 infectivity and to rule out any off-target effects of the inhibitors, we silenced FASN gene expression using specific short hairpin RNA (shRNA). Finally, the impact of these compounds on Abeta-like plaque formation was assessed using a 3D tissue culture model simulating herpesvirus-induced AD. Results. Here, we demonstrate that de novo lipogenesis is essential for HSV-1 infectivity. Specifically, HSV-1 infection upregulates FASN expression, accompanied by a marked increase in lipids and a differential lipid species distribution. Conversely, silencing FASN or applying FASN inhibitors CMS121 and C75 during infection reduces viral infectivity, affecting virion structure and entry into host cells. Additionally, we show that a source of lipid-rich external factors provided by fetal bovine serum significantly increases HSV-1 infectivity. Lastly, in a 3D tissue culture model of herpesvirus-induced AD, both CMS121 and C75 display a potent inhibitory effect on Abeta-like plaque formation, linking HSV-1-mediated lipid metabolism

dysregulation to AD etiopathogenesis. Discussion and Conclusions. Altogether, our findings reveal how HSV-1 manipulates lipid metabolism, offering insights into its association with chronic disease and therapeutic intervention.

77 - LACTOBACILLI-CONDITIONED MEDIA: ASSESSMENT OF CYTOTOXIC ACTIVITY AGAINST COLON CELL LINES

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Lactobacilli-conditioned media: assessment of cytotoxic activity against colon cell lines

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Introduction: Nowadays, probiotics, viable microorganisms that provide beneficial effects to the human host, are regarded as weapons to fight against various diseases such as autoimmune diseases like Crohn's Disease or other pathologies, primarily focusing on cancer. However, the "dark side of the moon" about probiotics is that, in many cases, they can cause side effects in various patients. For this reason, attention has shifted to parabiotics and primarily to postbiotics, which are probiotic-derived products like bacteriocins with potential applications in every branch of medicine. Material and methods: The aim of this study was to assess the cytotoxic activity of cell-free supernatant conditioned by two strains of Lactobacilli: L. plantarum and L. paracasei. Cell culture media were conditioned for 24 hours with bacteria in the late-exponential phase to enhance the secondary metabolism and thus the production of potentially active secondary metabolites. To evaluate the cytotoxic activity of the cell-free supernatant from L. plantarum (LP) and L. paracasei (LPC) MTT assay has been performed on CCD18-Co and HCT-116 cell lines. Results: The preliminary analyses have highlighted excellent cytotoxic activity even at low concentrations against the HCT-116 tumor cell line. Conversely, CCD18Co fibroblasts showed low susceptibility, indicating that the mode of action is selective against tumor cells. Discussion and Conclusions: These findings were further supported by the cytotoxic activity against intestinal tumoroids and the absence thereof against organoids derived from the same patients. These results suggest that the cell-free supernatant from L. plantarum and L. paracasei may offer selective cytotoxicity against cancer cells, indicating promising therapeutic potential.

119 - SEARCHING FOR NEW GUT FRIENDS: IN VITRO CHARACTERIZATION OF THE PROBIOTIC PROPERTIES OF HUMAN COLONIC ISOLATED ESCHERICHIA COLI.

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Searching for new gut friends: in vitro characterization of the probiotic properties of human colonic isolated Escherichia coli.

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Introduction. Inflammatory bowel diseases (IBDs) are chronic inflammatory condition of the gastrointestinal tract (GI), and include ulcerative colitis (UC) and Crohn's disease (CD) both characterized by an altered mucosa structure, systemic biochemical disorders due to intestinal altered permeability and extended modification of the gut microbial composition. Patients present reduced microbial diversity often in combination with a lower abundance of obligate anaerobic bacteria and an expansion of facultative anaerobic bacteria, particularly from the Proteobacteria phylum. Indeed, stable colonization by Escherichia coli pathotypes (e.g. Adherent Invasive E. coli (AIEC) or E. coli belonging to the B2 phylogroup) is frequently observed in patients suffering from IBDs. Then, the replacement of E. coli pathotypes with commensal strains is considered a valuable approach for the treatment of IBDs. Specific formulations of probiotics were shown to be effective if administrated in conjunction with standard therapies in patients suffering from IBDs and one of the most used probiotic is the E. coli strain Nissle 1917 (EcN). Although EcN possesses all the characteristics to be the ideal probiotic, the results in terms of contributing to/maintaining the remission of inflammatory diseases as well as preventing the relapse of the diseases are extremely variable affecting its efficacious applications. Hence, this project aimed at selecting several heterogeneous E. coli isolates collected from the colonic mucosa of healthy subjects to develop a new probiotic product enriched in commensal strains beneficial for IBD patients. Materials and Methods. From our previously described E. coli collection, genotypically different isolates sharing common phenotypic features were assayed for their probiotic properties including fitness-associated factors, such as (i) metabolic requirements (competitive capability to metabolize simple sugars and dietary proteins in comparison to different E. coli pathotypes), (ii) production of microcins and colicines and (iii) evaluation of competitive planktonic and sessile growth against selected E. coli

pathotypes. EcN was used as control. Results. Commensal E. coli isolates can be classified as faster or slower growing depending on the sugar metabolized in comparison to EcN. As EcN, 4 isolates were able to produce and release thermolabile molecules showing inhibitory activity against different E. coli pathotypes. Competitive planktonic and sessile growth indicated the capability of commensal E. coli isolates to restrict E. coli pathotypes growth. Conclusions. Our in vitro characterization demonstrates that intra-species competition represents an efficient strategy to reduce the burden of E. coli pathotypes. This feature could help the development of new E. coli-based probiotic formulations whose efficacy should be tested to improve the quality of life of patients suffering from IBDs.

135 - METAPROTEOMIC PORTRAIT OF THE HEALTHY HUMAN GUT MICROBIOTA

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METAPROTEOMIC PORTRAIT OF THE HEALTHY HUMAN GUT MICROBIOTA

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1. Introduction

Gut metaproteomics can provide direct evidence of microbial functions actively expressed in the colonic environments, contributing to clarify the role of the gut microbiota (GM) in human physiology. However, there is still a lack of knowledge about the "core" colonic metaproteome of a healthy human population. In this study, we re-analyzed 10 fecal metaproteomics datasets of healthy individuals from different continents and countries, with the aim of identifying stable and variable gut microbial functions and defining the contribution of specific bacterial taxa to the main metabolic pathways.

2. Materials and Methods

Following a search for publicly available fecal metaproteomics datasets containing healthy subjects, we re-analyzed 8 datasets, including cohorts from Australia, Canada, China, Germany, Italy, Spain, and USA. A previously published Italian study was extended with further 5 healthy subjects and two unpublished datasets were finally added, for a total of 10 datasets and 134 healthy human subjects. The same pipeline for peptide identification, quantification, and annotation, carried out using Proteome Discoverer, Unipept, eggNOG-mapper, and Meta4P as bioinformatic tools, was applied to the 10 datasets.

3. Results

The "core" metaproteome included 182 microbial functions and 83 pathways that were identified in all individuals analyzed. Several enzymes involved in glucose and pyruvate metabolism, along with glutamate dehydrogenase, acetate kinase, elongation factors G and Tu and DnaK, were the proteins with the lowest abundance variability in the cohorts under study. On the contrary, proteins involved in chemotaxis, response to stress and cell adhesion were among the most variable functions Faecalibacterium is the most stable genus and the top contributor to anti-inflammatory butyrate

production in the healthy GM. Active production of other mucosal immunomodulators facilitating host tolerance was observed, including Roseburia flagellin and lipopolysaccharide biosynthetic enzymes expressed by members of Bacteroidota.

4. Discussion and Conclusions

Our study provides a detailed picture of the healthy human GM, unveiling its functional mechanisms and its relationship with nutrition, immunity, and environmental stressors. For the first time, we detected co-occurrence and mutual exclusion dynamics involving bacterial taxa and functions, dissecting the taxon-specific contribution to molecular functions, biological processes and metabolic pathways actively expressed by the healthy human GM. These results encourage the use of fecal metaproteomics to investigate GM activity, opening the way to future multicentric studies with larger population cohorts and standardized analytical approaches.

154 - A 69.9-KB LONG INVERTED REPEAT GENERATES INSTABILITY IN A LABORATORY STRAIN OF LACTOBACILLUS CRISPATUS

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A 69.9-kb long inverted repeat generates instability in a laboratory strain of Lactobacillus crispatus

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Introduction: Long inverted repeats (LIRs), consisting of two inverted complementary copies of a DNA sequence longer than 100-bp, have been largely described in eukaryotes, but rarely reported in prokaryotes. In this study, the unusual LIRs of the Lactobacillus crispatus laboratory strain M247Siena, derivative of strain M247, were investigated.

Materials and Methods: Complete genome sequence of M247Siena was determined combining both Nanopore and Illumina sequencing technologies, whereas genome structure analysis was carried out by PCR genome mapping and by analysis of ultra-long (>80-kb) nanopore reads.

Results: Compared to the parental M247 strain, M247Siena genome contains an additional copy of a 69.9-kb long chromosomal segment, which replaced a 15.4-kb DNA segment. Both segments were flanked by the same insertion sequences (ISs), namely IS1201 and ISLcr2i, which probably mediated the recombination. The duplication resulted in two copies of the 69.9-kb long chromosomal segment, arranged as LIRs located 224.4-kb apart upstream and downstream the chromosomal origin of replication, respectively. Quantitative analysis of chromosomal rearrangements using Nanopore reads, indicated that the newly generated 69.9-kb LIRs of M247Siena increased its genomic instability. In fact, we detected two alternative chromosomal structures occurring at a frequency of 77% and 23% in our ultra-long nanopore reads. To investigate the origin of the duplication, we analyzed by PCR the occurrence of chromosomal rearrangements involving the 69.9- and 15.4-kb DNA regions in the M247 bacterial population, which was detected at a very low rate (2.19(±0.69) per 105 chromosomes).

Discussion and Conclusions: We identified chromosomal rearrangements likely associated with the presence of ISs, which might explain the origin a 69.9-kb LIRs in a laboratory strain of Lactobacillus crispatus, in turn we determined that the presence of this 69.9-kb LIRs in the M247Siena genome increased the genomic instability compared to the parental strain M247.

174 - PLANKTONIC AND BIOFILM MEMBRANE VESICLES RELEASED BY LIMOSILACTOBACILLUS REUTERI DSM 17938: BIOCHEMICAL AND FUNCTIONAL PROPERTIES

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Planktonic and Biofilm Membrane Vesicles Released by Limosilactobacillus reuteri DSM 17938: Biochemical and Functional Properties

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INTRODUCTION Limosilactobacillus reuteri DSM 17938 is among the most well studied probiotic strains and its safety and efficacy have been proven by numerous clinical studies. Nowadays, it is well established that probiotics can exert their beneficial effect on human health either through direct interactions with host cells or release of functional products, which are also delivered by Membrane Vesicles with both planktonic and biofilm phenotypes (pMVs and bMVs). Hence, the aim of the present study was (1) to characterize pMVs and bMVs via proteomic and metabolomic analysis (2) to evaluate

their toxicity in human cell models (3) to determine putative anticancer properties as well as modulation of macrophages. The toxicity in the Galleria mellonella in vivo model was also assessed.

MATERIALS AND METHODS L. reuteri MVs were isolated, and their size and concentration determined. The effect of MVs on cancer cells, namely Mz-ChA-1, HepG2, MDA-MB-468 and Bx-PC3, and non-cancerous cells, such as H69 cholangiocytes and murine RAW 264.7 macrophages, was evaluated. Cells were grown for 24h and then treated with serial dilutions of MVs. After 24 and 72h the cytotoxic effect was assessed by using the MTT assay, while the phagocytic abilities of macrophages were evaluated using the neutral red assay. To evaluate in vivo toxicity, 108 MVs/head were administered to G. mellonella larvae which were monitored for 4 days to score mortality. MVs were also characterized via proteomic and NMR-based metabolomic analysis.

RESULTS In Bx-PC3 cells, bMVs gave a 10 to 20% inhibition of viability starting from 108 bMVs/mL and pMVs showed early signs of cytotoxicity only at 109 pMVs/mL. Both types of MVs were non-cytotoxic on Mz-ChA-1 and HepG2 cells, while pMVs induced a 10% reduction of viability in MDA-MB-468 at the highest concentration. MVs were well-tolerated by non-cancerous cells and were able to affect phagocytosis in LPS-induced macrophages. In the in vivo model, MVs were non-toxic at the concentrations tested.

DISCUSSION AND CONCLUSIONS Both pMVs and bMVs from L. reuteri DSM 17938 showed no toxicity on non-cancerous cells and in the in vivo model. They weakly affected the viability of the cancerous cells, so it cannot be concluded that vesicles have antiproliferative properties. Nevertheless, the results obtained regarding LPS-induced macrophages indicate a possible immunomodulatory effect. NMR and proteomic analysis revealed a unique composition of both types of vesicles, suggesting that specific components may exert distinct functions closely related to strain and phenotype. These data demonstrate that L. reuteri MVs may be used as part of an immunomodulatory strategy and encourage new studies to further describe the correlation between composition and function.

175 - THE ENIGMATIC ROLE OF RID FAMILY PROTEINS AT THE INTERSECTION BETWEEN MICROBIAL PHYSIOLOGY, METABOLISM AND VIRULENCE

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The enigmatic role of Rid family proteins at the intersection between microbial physiology, metabolism and virulence

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The reactive intermediate deaminase (Rid) family is a large family of proteins widely distributed across all domain of life. Phylogenetic analysis allowed to split the Rid family members into eight subfamilies: RidA and seven other subfamily members (Rid1 to Rid7). While the function of RidA as an enamine/imine deaminase is well characterized, the function of the other Rid family members is poorly understood. Recently, a Rid7 family member, Rid7C, was involved in RNA processing in an actinomycete. Intriguingly, Rid7C, when overexpressed in E. coli, co-purified with large amounts of RNA.

We then analyzed different Rid proteins from Salmonella enterica (RidA, Rid2 and Rid7), Pseudomonas aeruginosa (RidA and Rid1) and Pseudomonas syringae (Rid3) overexpressed in E. coli and found that only S. enterica Rid7 but not the other Rid proteins co-purified with RNA. This finding prompted us to establish the physiological role of Rid7, and we therefore began to identify the RNA molecules bound to Rid7 by RNAseq.

We found that Rid7 co-purified with the RNA sponges ScoR and chb, and their respective small RNA targets GcvB and ChiX, as well as other regulatory small RNAs (GadY, GlmZ, OmrB and NarS) involved in metabolic adaptation, suggesting a role of Rid7 in processing of RNA complexes involving these RNAs. Comparative analysis of RidA, Rid2 and Rid7 sequences revealed the presence of the RfxxxH motif (where f is often N, D, or H, and x is any amino acid that can vary from 3 to 5 residues) in Rid7 from both S. enterica and E. coli. This short motif is present in higher eukaryotes and prokaryotes nucleotide-binding (HEPN) superfamily proteins, which is an emerging group of endoribonucleases found across all domains of life. The motif is located within a region of Rid7 that has the tendency to form a small □ helix, and is absent in RidA and Rid2, which is consistent with the lack of RNA binding activity in RidA and Rid2.

These results may help to understand the role of Rid proteins in bacteria and their involvement in the regulation of Salmonella metabolism, also in relation to the different microenvironments of the host.

230 - AGING AND GENDER: FUNCTIONS OF THE GUT MICROBIOTA

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Aging and gender: functions of the gut microbiota

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Introduction

Healthy aging is defined as “the process of developing and maintaining the functional ability that enables well-being in older age”. The maintenance of health in later life has become a major public health challenge. There is considerable individual variability in the aging process between men and women. Women live longer than men, consistent with lower biological ages as assessed by molecular biomarkers, but women are frailer and have worse health at the end of life, while men still perform better in physical function examinations. Here, we proposed to conduct an observational study in a model population of healthy longevity in Sardinia: the purpose of this project is to study gender differences in gut microbiota aging, identifying microbial biomarkers related to healthy aging or age-related diseases.

Materials and Methods

200 people aging between 50 and 100 years were recruited. An interview questionnaire was prepared for each subject to assess their medical history (anamnesis, Mini Mental State Examination, Cumulative Illness Rating Scale) and to study eating habits (food frequency questionnaire); blood samples were taken for metabolic and haematochemical profile analysis and for DNA extraction. DNA samples were subjected to methylation analysis using Illumina's Infinium MethylationEPIC Kit (850k BeadChips). A fecal sample was collected from each subject to characterise the gut microbiota both taxonomically, through DNA extraction for 16S rRNA gene sequencing for the construction of libraries to be sequenced using Next Generation Sequencing (NGS) technology on the HiScan-Illumina platform. and functionally, by metaproteomics approaches, through extraction of total proteins and their peptide fragmentation, followed by shotgun analysis in mass spectrometry using liquid chromatography tandem mass spectrometry (LC-MS/MS) technology.

Results

Operative Results

Currently, the data collected from interview questionnaires and dietary questionnaires, metabolic and hematochemical profile data have been computerised. 200 blood samples were collected from which DNA was extracted, concentrated, quantified and analysed for DNA methylation profiling. 175 fecal samples have been collected: DNA has been extracted and sequenced to provide gut microbiota taxonomy.

Expected Results

Cognitive, nutritional, metabolic, haematochemical profile data, DNA methylation analysis data, and taxonomic and functional gut microbiota characterisation data will enable association studies to identify nutritional-, gender- and/or microbiota-dependent features that affect healthy longevity, generating novel hypothesis of interventions to promote active and healthy aging.

T03 ONE HEALTH

9 - MICROPLASTICS BIODEGRADATION POTENTIAL OF BACTERIA ISOLATED FROM AQUATIC ENVIRONMENT OF THE ADRIATIC SEA

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Microplastics biodegradation potential of bacteria isolated from aquatic environment of the Adriatic Sea

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Introduction

Microplastics (Mps) smaller than 5 mm are increasingly documented in aquatic organisms and their trophic transfer along food chain is a growing concern for food safety. MPs not only provide a substrate for the adhesion and proliferation of bacteria, but also for various microorganisms that activate their degradation, particularly Pseudomonas spp. However, their MPs biodegradation potential needs to be further investigated. The aim of our study is to evaluate the biodegradation potential of bacteria isolated from aquatic environments through the development of microbiological screening methods easily reproducible in the laboratory.

Materials and Methods

P. aeruginosa ATCC 15692 and P. putida ATCC 47054 which previously demonstrated biodegrading power towards LPDE (low-density polyethylene) were used to standardize the screening protocol. The standardized protocol was then used to test 6 Pseudomonas strains isolated from seafood and identified with the Matrix Assisted Laser Desorption/Ionisation Time-Of-Flight (MALDI-TOF) mass spectrometry technique: 3 P. putida (from clams and mussels of Adriatic Sea and Norwegian salmon) and 3 P. aeruginosa strains (from cod and hake of Pacific and Atlantic oceans and anchovies of the Adriatic Sea). The technique adapted for the purpose is the Clear Zone Method based on the inoculation of the test strain on a plate into a medium containing plastic as the only carbon source. A

lysis halo around the inoculum is an indicator of the use of plastic by bacteria. Two different medium formulations were tested: one containing PEG (polyethylene glycol, the soluble form of polyethylene) and one containing LPDE particles (50-100 micron size class). The two media were tested with and without yeast extract (0.1%). After incubation at 25°C for two weeks, staining with Comassie blue was carried out and the bacteria that produced a blue area on the dark background were considered positive.

Results

The best medium for displaying the biodegradation halo was the one containing PEG and yeast extract. Of the 6 field strains tested with the standardized medium, two strains of P. putida and one strain of P. aeruginosa, originating from the Adriatic sea, gave positive results.

Discussion and Conclusions

A first appreciable result was the standardization of a simple and easily reproducible method in a basic microbiological laboratory to select bacteria with biodegradative potential against MPs. Furthermore, an important result is the identification of 3 Pseudomonas strains originating from the Adriatic Sea potentially capable of biodegrading Mps. The next step will be to confirm the biodegradation activity of the three strains on plastic strips in an aquatic environment reproduced on laboratory scale.

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29 - ONE-HEALTH GENOMIC SURVEILLANCE PLAN PROPOSAL FOR ARBOVIRUSES TRACKING AND CONTROL: A CASE STUDY OF WEST NILE VIRUS MONITORING IN ROMAGNA (ITALY) THROUGH WHOLE GENOME SEQUENCING

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One-Health Genomic Surveillance Plan Proposal for Arboviruses Tracking and Control: A Case Study of West Nile Virus Monitoring in Romagna (Italy) through Whole Genome Sequencing

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Background

Despite the existence of an established one-health approach to Arboviruses epidemiological surveillance, the implementation of a systematic genomic surveillance initiative, integrating human and animal data, is still fragmented in Italy. The purpose of this study is to investigate the contribution of Whole Genome Sequencing (WGS) in the integrated genomic characterization of West Nile virus (WNV) in target avian reservoirs, mosquito vectors and infected humans in Romagna (northeastern Italy).

Materials and Methods

The study was conducted in Romagna, northeastern Italy, between May and October 2023 involving patients with encephalitis/meningitis and healthy blood donors who tested positive for WNV during screening programmes. Mosquitoes were sampled with attractive traps, and resident and migratory birds were collected as of active or passive surveillance. Samples were screened with WNV-specific

PCR (Polymerase Chain Reaction) assays; positive samples were subjected to WGS by hybrid-capture-mediated target enrichment (Viral Surveillance Panel, Illumina). Assemblies and consensus sequences were obtained with Bowtie2 v.2.4.1 and BCFtools. Maximum likelihood trees for viral phylogeny were constructed with best nucleotide substitution model, 1000 ultrafast bootstrap replicates and midpoint rooted.

Results

Eleven WNV lineage 2 sequences were obtained (4 meningitis cases, 3 blood donors, 3 Culex pipiens and 1 Pica pica), with a coverage yield from 98.8 to 100%. Nucleotide and deduced aminoacid conservation were high, with an average identity of 99.82% and 99.92%. Mutations of virulent clade 2d involved in human neuroinvasiveness were identified. Obtained sequences clustered within the Central-Southern European clade with robust bootstrap support, forming a distinct cluster with other viruses isolated in Italy in 2021-2023.

Discussion and Conclusions

Our preliminary results provide an insight into an integrated genomic characterisation approach of WNV strains and potentially other Arboviruses endemic in the study area, a high-transmission-risk hotspot for its geoclimatic characteristics and its proximity to migratory birds breeding grounds in local wetlands. Continued surveillance, throughout future seasons, will support the tracking of arboviruses genetic diversity, evolution, emergence of new variants or introduction of genetically diverse strains. Our approach suggests the importance of adopting an interdisciplinary multistakeholder strategy to integrate the steps from clinical diagnosis to entomological and ornithological monitoring, up to epidemiological and genomic surveillance. Engaging both human and animal health sectors will facilitate a comprehensive characterisation of endemic and emerging Arboviruses to mitigate their impact on public health.

61 - ANTIMICROBIAL EFFICACY OF PUNICA GRANATUM LYTHRACEAE PEEL EXTRACT AGAINST ESKAPE PATHOGENS

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Antimicrobial efficacy of Punica granatum Lythraceae peel extract against ESKAPE pathogens

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Introduction: To date, bacterial infections represent a leading public health threat due to the rapid emergence of multi-drug resistant (MDR) bacteria, a crisis attributed to abuse of antibiotics and a lack of new drug development. In the last 20 years, natural extracts with biological activities attracted scientific interest. Following the One Health Approach, natural by-products represent a sustainable and promising alternative solution. The aim of the present study was to evaluate the antimicrobial activity of hydro-alcoholic pomegranate peel extract (PPE) against MDR microorganisms belonging to Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. “ESKAPE” group pathogens. Materials and methods: The antibacterial effect of the PPE against ESKAPE pathogens, was evaluated by in vitro antimicrobial assays using the agar diffusion and the tube dilution methods. To verify the effect of PPE on the bacterial fitness, time-killing assay with increasing concentration of PPE was performed. The effect of PPE on cell viability was also evaluated. The cytotoxic activity of the PPE was tested on HeLa and HEK-293 cells lines through MTT assay at different concentrations of PPE for 24h and 30h. The effect of PPE on intracellular ROS production was defined treating the HeLa cell line for 24h with different concentrations of PPE. To investigate the anti-hemolytic action of PPE, the protective effect of extract against the hemolytic activity of H2O2 solution on human-washed red blood cells was examined. Finally, to evaluate whether PPE has an antibacterial activity also in the eukaryotic cell system, invasion assays in HeLa cells with S. aureus reference strain and MRSA clinical strain were performed. Results: PPE showed effective antimicrobial activity against ESKAPE pathogens. The kinetics of bactericidal action of PPE highlighted that microbial death was achieved in a time and dose

dependent manner. High concentrations of PPE exhibited antioxidant activity, providing a protective effect on cellular systems and red blood cell membranes. For the first time, a significant intracellular antibacterial property of PPE was reported by its bactericidal and bacteriostatic effect against the Staphylococcal strains in the HeLa cell line. Discussion and Conclusion: Our results demonstrate that the PPE is able to inhibit the bacterial viability of reference and MDR strains belonging to the ESKAPE group. We demonstrated that the PPE can reach the intracellular environment revealing its antimicrobial action. Our study suggests that PPE could be considered a therapeutic agent alone or in conjunction with standard antibiotics against challenging infections sustained by ESKAPE pathogens.

74 - DETECTION OF BORRELIA BURGDORFERI IN OFF-SEASON QUESTING TICKS: LYME-CARRYING TICKS LIVE LONGER AND SPREAD FARTHER

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Detection of Borrelia burgdorferi in off-season questing ticks: Lyme-carrying ticks live longer and spread farther.

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Introduction
Ticks are widely distributed throughout the world and serve as hosts for numerous pathogens, making them important contributors to zoonotic infections. Borrelia burgdorferi is a bacterial species that causes an emerging zoonotic tick-borne disease known as Lyme disease. Transmission cycles of the pathogen involve multiple host groups and are strongly influenced by environmental variables. The occurrence of Lyme disease is highly seasonal and the annual appearance of cases in spring is modulated by the weather conditions of the preceding months. Our work aims to understand the behaviour of the ticks during the winter months in Emilia-Romagna (Northeast Italy) in order to identify the presence of Borrelia burgdorferi in ticks and define the risks of out-of-season transmission of Lyme disease.
Materials and Methods
Tick collection sites were distributed along the city of Bologna (Emilia-Romagna region), specifically in the northern Apennines and suburban hill areas. The ticks were all morphologically identified as Ixodes rinicus. Ticks were pooled by stage and sampling site, homogenized through scalpels and pestles and DNA was extracted using TANBead® Nucleic Acid Extraction Kit (Taiwan Advanced Nonotech Inc.). DNA were screened for Borrelia burgdorferi sensu latu by Real-Time PCR targeting the 23S rRNA gene. Pools that tested positive by Real-Time PCR were sequenced using Illumina-based 16S Metagenomics Sequencing (Arrow Diagnostics).
Results
Questing ticks were sampled regularly during the period from February 2 to February 13, 2024. During the sampling period, a max temperature of 15°C and a min temperature of 0°C, with a mean humidity of 88%, has been registered. 157 ticks were collected including 6 adults (4%), 5 larvae (3%) and 146 nymphs (93%) that were divided into 27 pools. The pools were tested in Real-Time PCR for detection of B. burgorferi sl DNA and 4 pools (15%) were resulted positive with a mean Cycle threshold (ct) of 30. 1

out of 4 pools, as a pilot sample, was sequenced identifying the presence of Borrelia burgdorferi sensu strictu, along with 140 other microorganisms.

Discussion and Conclusions

Warming winters are changing arthropod behaviour patterns and will have important consequences for the spread of vector-borne infectious diseases. Our results show a preliminary picture of the circulation of Borrelia burgorferi in the winter season, demonstrating the importance of considering off-season transmission cycles of the infection and the impact of climate change on life cycle of ticks. There is therefore an urgent need to turn the spotlight on the possibility of transmission of vector-borne diseases all over the year, improving the clinical identification of symptoms and microbiological diagnosis.

102 - ISOLATION OF MEROPENEM RESISTANT ENTEROBACTERIA FROM THE CHIENTI RIVER

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Isolation of meropenem resistant enterobacteria from the Chienti river

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Introduction. In recent years, the rapid spread of antibiotic resistant bacteria in environmental ecosystems has raised great concern. Among them, extended-spectrum β-lactamase and carbapenemase-producing enterobacteria are considered high-priority pathogens on the global list of antibiotic-resistant bacteria (ARB) by the World Health Organization (WHO). Limited information is available on their diffusion in Italian environments, and particularly in surface waters. The aim of this study was to characterize carbapenem-resistant isolates retrieved from the Chienti river (Marche region, Italy), during a collection of ESBL-producing Enterobacteriaceae.

Materials and Methods. In September 2023, water from the Chienti River was sampled three times at four different sites to analyse the presence of cefotaxime-resistant Enterobacteriaceae. Isolation was carried out on Chromogenic coliform agar (bioMerieux, France) supplemented with 1 mg/mL of cefotaxime. Identification was conducted using the API20E system (bioMerieux, France). Antibiotic susceptibility testing was carried out using the disk diffusion assay according to EUCAST Guidelines and the combination disc test was performed using Neo-Sensitabs (Rosco Diagnostics). Antibiotic resistance genes (blaCTX-M, blaNDM, blaKPC, blaTEM, blaSHV, qnrA, qnrS, qnrB, sul1, sul2, sul3), and intI1 gene were detected by PCR and, when appropriate, were sequenced.

Results. Out of 25 cephotaxime resistant Enterobacteriaceae recovered from the 12 water samples collected from the Chienti river, six meropenem resistant isolates were found: two Citrobacter freundii, two Klebsiella pneumoniae, one E. coli and one Enterobacter cloacae. In four isolates, the meropenem resistance was attributed to the blaKPC gene, and in the other two to the production of the metallo beta lactamase NDM. These data were confirmed by the phenotypic test. Among the bacteria investigated in this study, which were all resistant to levofloxacin and carried the blaTEM gene, three isolates out of six carried the intI1 integron, a marker of their anthropogenic origin.

Discussion and Conclusions. The presence of carbapenem resistant pathogens provides strong evidence that river water could act as a huge reservoir of these AMR bacteria, potentially threatening human health. In addition, these findings underline the need of a permanent monitoring of surface water and the application of rigorous and efficient treatments of wastewaters.

108 - DETECTION OF ANTIBIOTIC-RESISTANCE AND GENETIC ANALYSIS OF ESCHERICHIA COLI ISOLATED FROM ALGERIAN DOGS USING LONG-READS APPROACH (MINION PLATFORM)

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Detection of antibiotic-resistance and genetic analysis of Escherichia coli isolated from algerian dogs using long-reads approach (MinION platform)

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Introduction

Antibiotic resistant Escherichia coli is becoming a major public health problem in Algeria. The role of companion animals (like dogs) in the epidemiology of antibiotic-resistant E. coli is an important field of study, even though most researchers examined closely the antibiotic resistance in humans and cattle. This study focused on the detection of antibiotic resistance in E. coli using long-reads whole genome sequencing (WGS) and reference-based assembly of E. coli strains isolated from healthy dogs in Algeria.

Materials and Methods

Fifteen E. coli strains were isolated from canine faecal samples. Phenotypic identification was obtained with VITEK® 2. The disk diffusion test was used to assess the resistance profile of each strain against the following antibiotics Ceftadizime (CAZ), Cefotaxime (CTX), Aztreonam (ATM), Cefepime (FEP), Amoxicillin+clavulanic acid (AMC), Gentamicin (CN), Amikacin (AK), Kanamicin (K), Tetracycline (TE), Cafazolin (CZ), Chloramphenicol (C), Amoxicillin (AMX), Nalidixic acid (NAL), Levofloxacin (LEV), Ciprofloxacin (CIP) and Imipenem (IMP). DNA was extracted using the Quick-DNA™ HMW MagBead Kit (Zymo Research, Irvine, CA, USA). The sequencing libraries were prepared using the rapid barcoding sequencing kit (SQK-RBK114.24). Twelve barcoded samples were loaded in a MinION FLO-MIN114 R10.4.1 flow cell (Oxford Nanopore Technologies Ltd.) and sequenced in a MinION Mk1C for 72 h. The fast5 files were basecalled with Dorado Basecall Server 7.1. Fastq raw data were analysed using the Automatic Bacterial Isolate Assembly, Annotation and Analyses (ASA3P) Pipeline using the E. coli K12 reference genome. Assemblies were analysed with different pipelines to derive pivotal information about antibiotic resistance (Abricate v1.0.1), phylogeny (CSIPhylogeny v1.4), MLST (MLST v2.0.9), and plasmids (PlasmidFinder v 2.0.1).

Results

AK, CN, C, and IMP were the most effective antibiotics, while all the strains were resistant to CTX, CZ, and AMX. The following beta-lactamases were identified: AmpC (100%), bla TEM-1 (60%) and bla CTX-M-15 (33.3%). All the strains carried for at least two different active efflux pumps (emr, mdf, mdt). Genetic metrics obtained from the sequencing showed that the GC% was 50.45% and the mean genome length was 5.77 Mbp with a N50 of 5 kbp. Five different MLST profiles were detected ST457

(13.3%), ST117 (26.6%), ST354 (6.6%), ST90 (6.6%), ST405 (6.6%). The following plasmids were found IncFIB (100%), IncFIC (73.3%), IncI1-I(Alpha) (26.6%), IncN (26.6%).

Discussion and Conclusions

This study shed light on the current epidemiology of antibiotic-resistant E. coli in algerian dogs thus supporting the One Health strategy against antibiotic resistance.

109 - CHARACTERISATION OF THE BACTERIAL COMMUNITY STRUCTURE OF OFFSHORE MEDITERRANEAN SEAWATER SAMPLES

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Characterisation of the bacterial community structure of offshore Mediterranean seawater samples

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Introduction

Microbial diversity affects the stability of ecosystem functions, as microbial communities play a key role in the dynamic biogeochemical structuring of the oceans. Marine ecosystems are highly dynamic and can be strongly influenced by climate change and pollution with direct and indirect effects on human health. While the effects of anthropogenic activities on coastal areas have been extensively studied, our understanding of their impact on the open sea is limited. To this end, a first overview of the composition of the surface bacterial communities of the Mediterranean Sea is provided from the first phase of a larger investigation, the Sea Care project, a joint initiative between the Italian Navy and the National Institute of Health. This broader initiative encompasses all the world's oceans, looking to identify emerging contaminants and assess associated health risks.

Material and Methods

Prefiltered (5.0 µm) water samples (n= 24; 3-5L) from the southwest (MED SW) and northwest (MED NW) Mediterranean Sea were filtered through 0.2 µm membrane filters, and total genomic DNA was extracted using the DNeasy PowerWater Kit (Qiagen). The V3-V4 variable region of the 16S rRNA was amplified using the MiSeq rRNA amplicon sequencing protocol (Illumina). The Chao1 alpha diversity index was used to calculate bacterial richness and the Shannon index to estimate bacterial evenness (QIIME2).

Results

The phyla Proteobacteria, Cyanobacteria, Bacteroidota and Actinobacteriota were found in 100% of the samples with the following range of relative abundance: 42.5-93.7%, 0.3-46.2%, 0.2-9.8% and 0.1-18.6%, respectively.

SAR11 clade Ia, Synechococcus CC9902 and AEGEAN 169 were the most ubiquitous genera detected in 100% of the samples, with relative abundances ranging from 1.2 to 36%, from 3% to 45.6% and from 0.16 to 10.8%, respectively. Alteromonas was present in 83.3% of the samples (relative abundance: 0.2-81.2%), Idiomarina in 70.8% (0.1-46.0%) and Pseudoalteromonas in 45.8% of the samples (0.12-6.5%). Genus Vibrio was detected (29% of the samples), as well as other genera associated with waterborne human and animal diseases (Pseudomonas, Acinetobacter, Streptococcus).

The alpha diversity (Chao 1 and Shannon indices) of bacterial communities at the genus level showed higher richness in MED NW than in MED SW samples.

Discussion and Conclusions

The maintenance of bacterial diversity in natural ecosystems is crucial for the function, stability and resilience of ecosystem processes. Our metagenomic investigation provides a first set of baseline data on the structure and diversity of bacterial communities in the Mediterranean Sea, in order to assess possible correlations with anthropogenic contaminants in subsequent phases of the project, as well as long-term impacts on human health from a One Health perspective.

171 - GLOBAL SPREAD OF ANTIBIOTIC RESISTANCE GENES ACROSS THE OCEANS AND SEAS: EVIDENCE FROM THE ATLANTIC, MEDITERRANEAN, PERSIAN GULF, RED SEA AND ARCTIC

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Global spread of antibiotic resistance genes across the oceans and seas: evidence from the Atlantic, Mediterranean, Persian Gulf, Red Sea and Arctic

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Introduction Antimicrobial resistance (AMR) is a major global health challenge, affecting both human health and the environment. The marine ecosystem has emerged as a critical reservoir of antibiotic resistance genes (ARGs), and although AMR is a long-standing natural occurrence, elevated levels often correspond to increased human activity. This study examines the distribution and co-occurrence patterns of ARGs in in the global oceans with the aim of assessing the potential anthropogenic impact of environmental factors.

Material and Methods Forty-three marine water samples (5 liters each) were collected between 2022 and 2023 from diverse seas and oceans: Mediterranean Sea, Persian Gulf, Gulf of Oman, Gulf of Aden, Red Sea, Atlantic Ocean, and the Arctic Ocean. The genes targeted for analysis were selected based on their known resistance to different classes of antibiotics: beta-lactamases (blaOXA48, blaCTXM1 group, blaTEM), sulfonamides (sul1), and tetracycline (tetA). The abundance of the ARGs was quantified by digital dPCR using the QIAcuity pentaplex instrument from Qiagen. To optimise a multiplex (pentaplex) assay for the five genes, a validation phase was performed by comparing the results with those obtained from individual assays. The abundance of ARGs was measured as both absolute and relative (normalised to 16S rRNA) gene copies/L to account for variations in bacterial load. To assess the similarities in ARGs across the samples, a clustering technique was used.

Results In total, 40/43 (93%) samples were positive for one or more ARGs. The mean concentrations (g.c/L) of 16S rRNA and ARGs were as follows: 16S rRNA (8.5 × 1014); blaOXA48 (1.5 × 109); blaCTXM1 group (9 × 109); blaTEM (4 ×109); sul1 (9.8 × 109) and tetA (1.2 × 109). The results show that sul1 was

widespread and reached ubiquitous levels, while the blaCTXM1 group was occasionally detected, mainly in the Arctic Ocean. Furthermore, in the Mediterranean Sea, a higher frequency of samples contaminated with multiple genes was observed, with several samples showing the presence of all five studied genes. Notably, also in the Arctic, specifically around the Svalbard Islands, a sample showed contamination by all the 5 ARGs investigated, suggesting widespread dissemination even in remote Arctic regions. In addition, clustering often grouped samples from diverse seas and oceans, indicating similar gene content (both qualitative and quantitative) among samples from different locations.

Discussion The results demonstrate regional variation due to different levels of anthropogenic impact and environmental factors, even in remote environments such as the Arctic. These results highlight the complexity and extent of the spread of antibiotic resistance genes in marine ecosystems, suggesting a significant interaction between human activities and the marine environment.

This project was made possible by the financial support of the "Sea Care Project: Health, Environment and Climate Research in the Vision of Planetary Health", a joint initiative between the Italian Navy and the National Institute of Health (Istituto Superiore di Sanità - ISS), with the main contribution from the Italian Navy. We also thank the European Union - NextGenerationEU in the framework of the National Recovery and Resilience Plan (NRRP) PE13 INF-ACT.

195 - AN INTEGRATED ONE HEALTH APPROACH: THE IMPACT OF STRAY ANIMAL POPULATIONS ON PUBLIC HEALTH IN NAPLES, ITALY.

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An integrated One Health approach: the impact of stray animal populations on public health in Naples, Italy.

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Abstract

Introduction. The rise of antimicrobial resistance poses a significant public health challenge, with an increasing number of organisms developing resistance to many antimicrobial agents used to treat infections in humans and animals. This study investigated the role of stray dogs and cats as sentinel animals in the urban environment. Therefore, the sampling of healthy skin was performed to isolate bacterial species present and assess their antimicrobial resistance. Materials e methods. A total of 117 stray dogs and 113 stray cats, were recruited for this study during the period between January 2022 - January 2024. Swabs were taken from healthy skin, precisely from back of the nose, nasal cavity, and ear of each animal hosted at the ASL NA 1 authority for sterilization as part of the efforts to control the stray dog and cat population in the urban area. All samples were plated using different culture agar media for the isolation of Gram-positive and Gram-negative bacteria. Then, bacterial identification was performed by MALDI-TOF-MS. The antimicrobial susceptibility testing, by using 19 and 18 antibiotics, for Gram-positive and Gram-negative bacteria, respectively, was performed. Results. A total of 690 swabs were collected, comprising 351 cutaneous swabs from dogs and 339 from cats. Of these, 31% of canine and 29% of feline swabs tested negative. A total of 770 bacterial strains were classified in 381 Gram-positive bacteria (94%) and 24 Gram-negative bacteria (6%) from dogs, and 355 Gram-positive bacteria (97%) and 10 Gram-negative bacteria (3%) from cats. Among the isolated Gram-positive bacteria in dogs, Staphylococcus pseudintermedius was the most prevalent, with 71 strains identified. Additionally, among the isolated Gram-negative bacteria, in both animal species, E. coli was predominant. In cats, coagulase-negative staphylococci were the most frequently isolated bacteria, with Staphylococcus felis being the predominant species. The phenotypic antibiotic resistance profiles revealed that over 75% of the canine Gram-positive strains exhibited resistance to cefoxitin, oxacillin, and penicillin, indicating probable methicillin-resistant strains; while for the feline Gram-positive strains over 75% of resistance was observed only against cefoxitin. For canine Gram-negative bacteria, resistance >80% for amoxicillin-clavulanate, spiramycin, penicillin and metronidazole, and in cats, resistance >80% for metronidazole, spiramycin and penicillin was observed. Conclusion. An update on antibiotic resistance profiles of both Gram-positive and Gram-

negative bacteria commonly present on the skin of stray animals is crucial for strengthening stewardship programs in public veterinary hospitals, aligning with the One Health approach.

This research was approved by the Ethical Animal Care and Use Committee of the University of Naples Federico II (Approval N. PG/2020/0057490 del 13/07/2020) and performed following the ethical precepts of the Declaration of Helsinki.

197 - QUASISPECIES ANALYSER (QSA) AS A NOVEL BIOINFORMATIC TOOL FOR THE ANALYSIS OF VIRAL POPULATION GENETICS

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QuasiSpecies Analyser (QSA) as a novel bioinformatic tool for the analysis of viral population genetics

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Introduction

High mutation rates characteristic of quasispecies viruses give rise to a population structure known as genomic cloud, where several variant genomes co-exist inside the infected host. The increasing availability and use of High-Throughput Sequencing (HTS) with its depth of coverage potentially yields additional relevant information on intrinsic sequence heterogeneity, compared to standard Sanger sequencing. Current bioinformatic research mainly focuses on the consensus sequence obtained by averaging the variant viral genomes found in biological samples. However, this kind of analysis is insufficient, as all the information regarding the viral population structure is lost in the process. In order to overcome this obstacle, a novel bioinformatic tool has been developed. QuasiSpecies Analyser (QSA) is a software able to utilize previously discarded information directly from the output of HTS techniques.

Materials and Methods

Starting from a set of HTS reads of a defined viral target in a sample, QSA is able to: (i) calculate the Position-Specific Scoring Matrices (PSSMs) for each sample; (ii) calculate the efficiency (normalised Shannon's entropy) values for each position in the amplified region of interest; (iii) retrieve an intra-sample diversity score based on the efficiency values of the sample, referred to as alfa-diversity; (iv) compute an inter-sample diversity score based on the efficiency values of two samples, called the delta-diversity.

Results

At the present moment, QSA has been tested on Human Parvovirus B19 (B19V), but its features can be applied to any quasispecies viruses. With QSA it has been possible to recognize the regions of high mutations and to distinguish them from higher conserved regions. alfa- and delta-diversity values have also been successfully utilized to describe the intra-sample variability and inter-sample diversity.

Discussion and Conclusions

Further development of QSA will allow it to perform additional kinds of analyses, such as haplotypes detection with the use of Hidden Markov Models (HMM), which are extensively used to this end. QSA will provide its users with a responsive Graphical User Interface (GUI), enabling any researcher to use it for their research on quasispecies viruses.

213 - A SNAPSHOT OF MICROBIOME OF AN ADULT POPULATION WITH HIGH-RISK BLADDER CANCER AND CORRELATION WITH THERAPEUTIC RESPONSE.

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A snapshot of microbiome of an adult population with high-risk bladder cancer and correlation with therapeutic response.

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Introduction. Patients with bladder cancer (BC) are usually treated with transurethral resection of bladder tumor (TURBT), in addition to adjuvant intravesical therapy (chemotherapy or anti-cancer immunotherapy with Bacillus Calmette Guerin- BCG) for those at intermediate-risk and high-risk of recurrence and progression. More recently, many reports are emerging about the role of the urinary microbiome in urothelial carcinogenesis, including gender disparity in bladder cancer and response to treatment.Aim of our study was to evaluate the different composition of microbiome in resistant and responder patients to BCG treatment.

Materials and Methods. A retrospective analysis was conducted including 31 patients (15 resistant and 16 responders to treatment with BCG), with high-risk bladder cancer treated with BCG. Bladder cancer tissues were collected pre-treatment, and formalin-fixed paraffin-embedded (FFPE) samples were analyzed. DNA was extracted from FFPE samples and microbiome was evaluated by amplification of 16S rRNA targeting the V1-V3 regions. Chi-square and Fisher's exact tests were assessed for evaluation of the differences between groups.

Results. Considering the mean/median percentages of the presence of a specific phylum for each group, we observed a significant highest mean/median percentages of presence for Firmicutes and Verrucomicrobiota in responder than resistant patients (median: 1.1 vs 0.3, p=0.0293 and 0.9 vs 0.1, 0.0285, respectively). In addition, considering the percentage of patients with a specific phylum, we found a significant more presence of Fusobacteriota in responders than resistant (75% vs 33.3% , p=0.0198), vice versa for Cyanobacteria (31.3% vs 73.3%, p=0.0191).

Conclusions. These preliminary data showed the presence of significant difference for some phyla between two groups, suggesting the possible of the role of microbiome in bladder cancer and in the response to therapy. The microbiome analysis could direct physician to more personalized treatments and targeted therapeutic interventions.

232 - GUT MICROBIOTA ALTERATION CONTRIBUTES TO CARDIAC DYSFUNCTION INDUCED BY PARTIAL GENETIC DELETION OF AKAP1

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Gut microbiota alteration contributes to cardiac dysfunction induced by partial genetic deletion of Akap1

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1. Introduction: Down-regulation of mitochondria-targeted A-kinase anchoring proteins (D-AKAP1) encoded by the Akap1 gene promotes pathological cardiac hypertrophy and heart failure. In this study, we explored the microbiota-gut-heart axis by first studying the influence of AKAP1 levels on gut microbiota (GM) composition. Subsequently, through fecal microbiota transplantation (FMT) experiments, we investigated the effects of the microbiota profile of AKAP 121-deficient mice on the microbiota-heart axis and heart functionality. 2. Materials and Methods: Young (4-6-month-old) and old (18-24-month-old) Akap1+/+ and genetically modified Akap1+/− mice were evaluated for cardiac function by echocardiography, gut barrier function by mRNA analysis of Occludin (Ocln) and Tight junction protein ZO-1 (Tjp1) and systemic inflammation by measuring circulating levels of selected cytokines and Lipopolysaccharide (LPS). High-throughput sequencing of V3–V4 regions of the 16S rRNA gene followed by QIIME2 bioinformatics analysis were employed to assess fecal microbiota structure and composition. Moreover, LEfSe was used to find significant bacteria associated with each group of animals. The impact of GM on cardiac functionality was explored by transferring feces from Akap1+/- donors mice to Akap1+/+ recipient mice and vice versa; the effects of FMT on gut barrier function, microbiota composition, inflammation and cardiac function were determined. 3. Results: Akap1 partial deletion impaired gut barrier function as shown by reduced colon levels of Ocln and Tjp1, increased circulating levels of LPS, TNF-alpha, IL-1, reduced levels of IL-10, and induced early cardiac systolic dysfunction in young and old Akap1+/− mice compared to Akap1+/+. Compositionally distinct metacommunities were found in Akap1+/- mice with a more severe degree of microbiota alteration in the old mice subgroup. Interestingly, the reciprocal exchange of fecal microbiota affected gut permeability and cardiac function in recipient mice. 4. Discussion and Conclusions: The aberrant

GM profile induced by Akap1 partial deletion leads to abnormalities of gut homeostasis and contributes to cardiac dysfunction pathophysiology in Akap1+/- mice. FMT results suggest a causative role of gut microbial communities on systemic inflammation, intestinal integrity and possibly cardiac dysfunction in mice carrying Akap1 partial deletion. These results may allow the identification of innovative fecal and tissue biomarkers and provide new insights for elucidating the processes underlying the microbiota-gut-heart axis.

240 - TTV AND CMV VIRAL LOAD DYNAMICS: WHICH EMERGES FIRST DURING IMMUNOSUPPRESSION?

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TTV and CMV viral load dynamics: which emerges first during immunosuppression?

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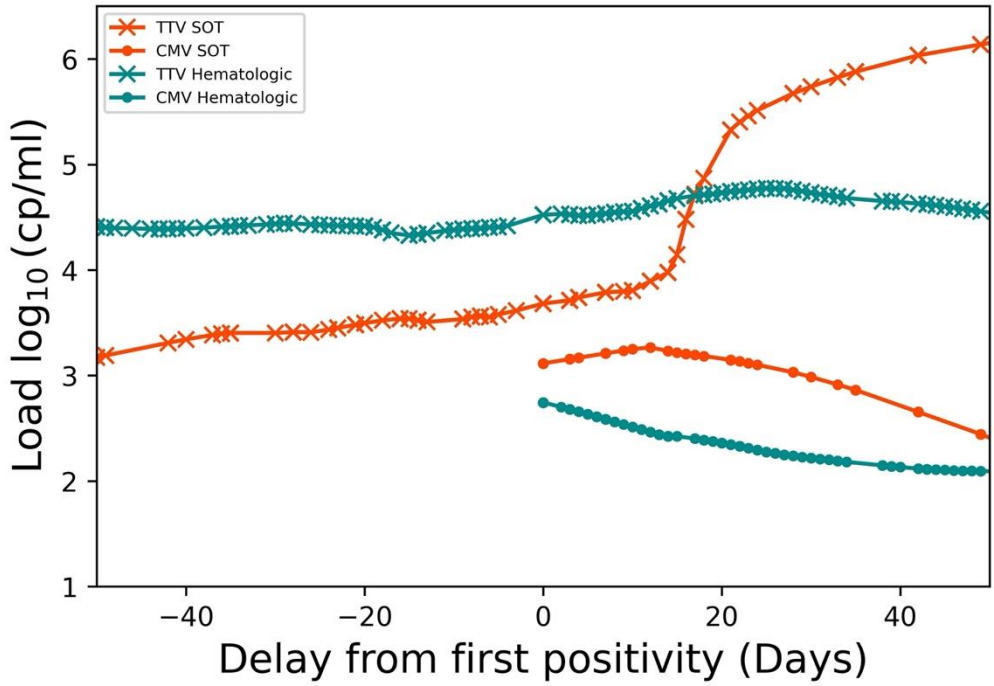
Introduction:Novel biomarkers reflecting the degree of immunosuppression in transplant patients are required in order to ensure eventual personalized equilibrium between rejection and infection risks. Materials and Methods: With the above aim, Torque Teno Virus (TTV) viremia was precisely examined in a large cohort of transplanted immunocompromised patients (192 hematological and 60 solid organ transplant recipients) being monitored for Cytomegalovirus (CMV) reactivation. Results:TTV load was measured in 2,612 plasma samples from 448 patients. The results revealed a significant increase in TTV viral load approximately 14 days following CMV reactivation in SOT patients (Figure). No recognizable difference in TTV load was noted among hematological patients during the entire timeframe analyzed. Furthermore, a temporal gap of approximately 30 days was noted between the

viral load peaks reached by the two viruses, with TTV preceding CMV. Discussion and Conclusion:It was not possible to establish a correlation between CMV reactivation and TTV viremia in the hematological patients. On the other hand, the SOT patient cohort allowed us to analyze viral kinetics and draw intriguing conclusions. Taken together, the data suggest, to our knowledge for the first time, that CMV infection itself could potentially cause an increase in TTV load in the peripheral blood of patients undergoing immunosuppressive therapy.



255 - DETECTION OF INFLUENZA VIRUSES IN WASTEWATER: A SYSTEMATIC LITERATURE REVIEW FOR METHOD EVALUATION

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Detection of influenza viruses in wastewater: a systematic literature review for method evaluation

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Introduction: The detection of pathogens in wastewater, an approach commonly known as Wastewater-Based Epidemiology (WBE), is a “One Health” method for monitoring the spread of a disease in a community, including both symptomatic and asymptomatic individuals. Using this approach for influenza viruses (IVs) could serve to describe the intensity and timing of seasonal outbreaks, and potentially report the emergence of new viral subtypes. With the aim of identifying the best methodologies for establishing a wastewater-based surveillance system in Lombardy (Italy), we conducted a systematic review of WBE experiences described in the academic and grey literature.

Methods: The study protocol for this systematic review was registered on the PROSPERO website (registration ID: CRD42024532435). Studies were retrieved from three databases: PubMed/Medline, Web of Science and Scopus. Eligibility criteria were defined as follows: studies reporting measurements of IV concentration in human wastewater or describing genetic characterization in the same matrix were included, while those describing environmental monitoring in an animal/avian context were excluded. The following data were extracted: sampling location and frequency, type of matrix sampled (liquid vs. solid), detection and normalization methods used, quantitative measures of IVs in wastewater, strength of correlation between environmental concentrations and cases of influenza in the corresponding community, general aim of the study.

Results: The database search was initiated on 8 April 2024, and resulted in the retrieval of 815 records, of which 449 were eliminated at the de-duplication stage. The remaining 366 articles were screened for title and abstract content, resulting in the identification of 50 potentially relevant studies. A second phase of screening, which consists in reading the full text of selected papers to confirm their inclusion in the review, and extracting relevant data, is currently underway.

Discussion and conclusions: The systematic review of the scientific literature in the field of WBE serves to identify which methods (including pre-treatment, concentration, nucleic acid extraction, quantification, normalization, etc.) have been used for the detection of IVs in wastewater, and to assess their effectiveness in terms of the strength of correlation with data acquired through clinical

surveillance systems (e.g. number of infected individuals). The best performing methods identified will be experimentally evaluated in the laboratory, with the ultimate goal of establishing an effective protocol for future environmental monitoring campaigns of IVs in Lombardy.

280 - USE OF ESSENTIAL OILS IN INTENSIVE RABBIT FARMS TO REDUCE THE USE OF ANTIBIOTICS.

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Use of essential oils in intensive rabbit farms to reduce the use of antibiotics.

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Introduction

Rabbits are one of the most recently domesticated animals in the world. In the XX century rabbits graduated from outdoor pets to domesticated pets like cats or dogs. The study of the antimicrobial action of essential oils in the veterinary field in recent years has undergone an important growth reaching interesting results both for prevention and treatment of bacterial and fungal infections. The spread of such infections in animal husbandry poses an important health problem for operators because of the potential zoonotic infection that such microorganisms can cause. Specifically, dermatophytosis (diseases caused by dermatophytic fungi) is an extremely relevant problem in commercial rabbit farms where this fungi can cause considerable morbidity and economic losses in terms of animals. The most common cause of these zoonotic infections, responsible for tinea corporis, tinea capitis or tinea barbae-type dermatophysis, are caused by fungi such as Trichophyton spp. and Microsporum canis.

The objective of this study is to evaluate the effectiveness of essential oil (Eo) spray for the control of fungal load in rabbit farms in order to conteract the spread of dermatophytosis in a One Health bigger aim.

Material and Methods

The antifungal characterization began with antimicrobial susceptibility testing, performed with broth microdilution method, according to EUCAST-AFST guideline version E.Def 11.0. The antifungal effectiveness was tested for commercial mix of OE and for the individual EO that characterized the mix. The tested fungi were Microsporum canis, Trichophyton mentagrophytes and Trichophyton interdigitale. Minimum inhibitory (MIC) and fungicide (MFC) concentration values were extrapolated

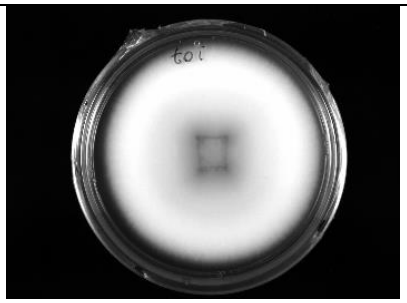
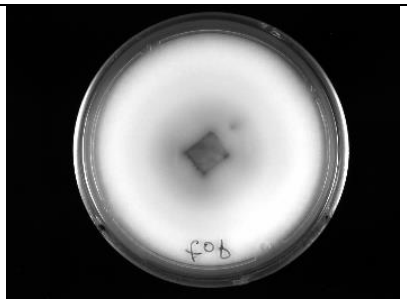
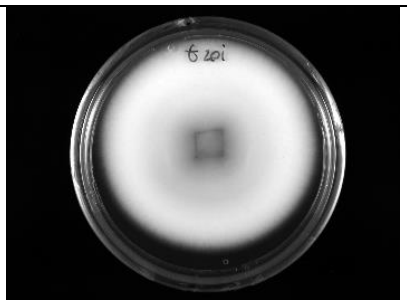
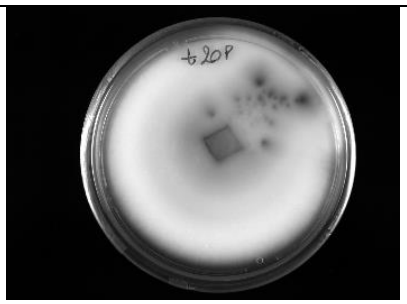
from the tests. The cytocidal efficacy of the volatile components of the mix was evaluated by spraying experiments in confined environments. Sterile paper's squares (1x1 cm) were inoculated at the time and 10 days before the experiment with 50 microliters inoculum at 5x105 cfu/ml. Two spraying times (20 and 80 min) were tested. After treatment the growth of dermatophytes was observed after 3, 6 and 10 days. After this time, the samples were then incubated in a Cytation 5 Cell Imaging Multi-Mode Reader with image acquisition every 3 hours for 60 hours. Finally, the samples were observed with scanning electron microscope (SEM) at 300x, 1000x and 10000x magnification.

Results

The results show that volatile components of EO exert a cytostatic action against the dermatophytes tested compared to the untreated control (Fig. 1). The figure 2 show fungi growth compared to untreated control after 60 hours of incubation at 30°C (Fig. 2). Finally, the results highlight an important difference in fungal biomass between control and treated samples (Fig. 3).

Discussion and Conclusions

Preliminary data suggest that the spraying of EO administered may be a valuable treatment for controlling fungal load in rabbit farms in order to avoid both animal and operator infections.

	Inoculated the same day	Inoculated 10 days before
Squares whitout treatment		
Squares treatment 20 minutes		

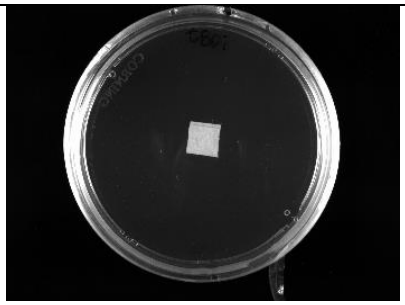
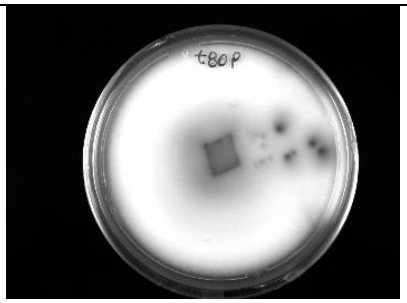
Squares treatment 80 minutes		

Figure 1 Paper's squares inoculated with Trichophyton interdigitale 10 days after treatment.

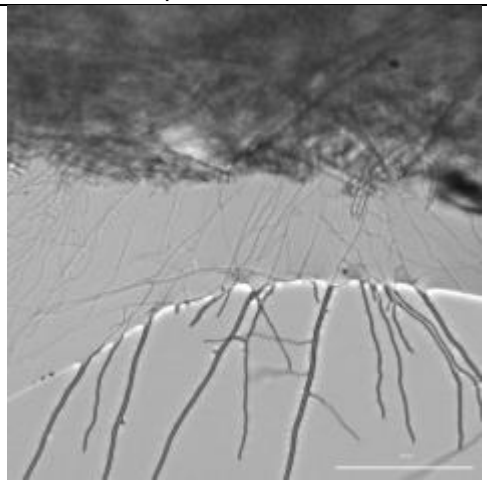
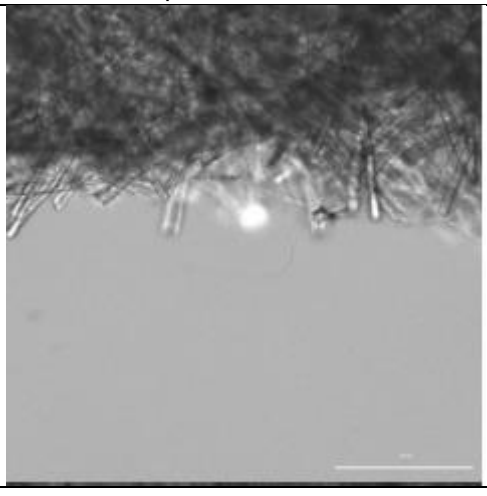
	Control sample	Treated sample
After 60 hours at 30°C		

Figure 2 Bright field images of paper's squares inoculated with Trichophyton interdigitale.

Control sample	Treated sample

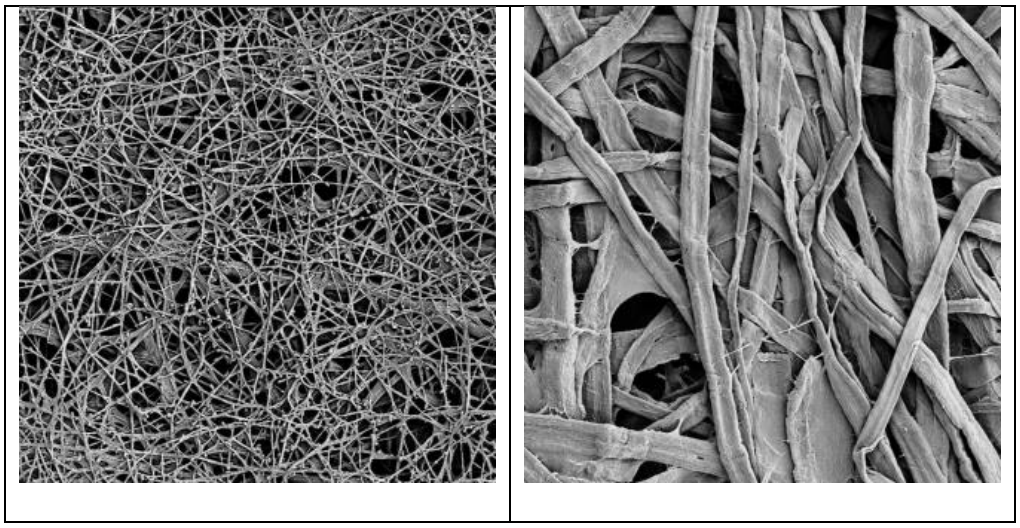


Figure 3 Micrographs of control and treated samples obtained with SEM with a magnification of 1000X and 15kW acceleration voltage.

283 - NATIONAL PHD PROGRAMME IN ONE HEALTH APPROACHES TO INFECTIOUS DISEASES AND LIFE SCIENCE RESEARCH: AN INNOVATIVE INITIATIVE FOR FUTURE OF MICROBIOLOGICAL RESEARCH

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Topics 2024: One Health

Title: National PhD Programme in One Health approaches to infectious diseases and life science research: an innovative initiative for future of microbiological research

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Abstract:

Introduction

The current global landscape, marked by the emergence and re-emergence of infectious diseases, underscores the critical need for an integrated approach to managing health crises. The INF-ACT project, funded by the European Union under the NextGenerationEU-MUR PNRR Extended Partnership

on Emerging Infectious Diseases (project number PE00000007), serves as a vital platform for addressing such challenges. From this project, the National PhD Programme in One Health approaches to infectious diseases and life science research derives as coordinated by the University of Pavia within a broad consortium of universities and research institutions across Italy.

Materials and methods

The PhD programme aims to foster a generation of researchers equipped with the skills to operate in an interconnected scientific context, transcending traditional academic disciplinary boundaries. Goals include the development of innovative strategies for surveillance, diagnosis, and treatment of infectious diseases, and the formulation of effective interventions against public health emergencies. The programme structures its activities around five main research nodes: i) emerging and re-emerging viruses, ii) arthropod vectors and vector-borne pathogens, iii) antimicrobial resistance, iv) epidemiology, monitoring, and modelling, and v) new therapeutic strategies.

Results

The call for admissions recorded significant participation, with over 120 applications from various Italian and international education. Of these, 50 researchers were selected to work in collaboration with INF-ACT partners. This group of PhD students, with diverse but complementary scientific backgrounds, forms a varied team committed to promoting the One Health approach. The faculty includes numerous professors from the MED/07 and BIO/19 scientific sectors. Research institutions such as Istituto Superiore di Sanità and Associazione Istituti Zooprofilattici Sperimentali also made crucial contributions during the recent pandemic, laying the groundwork for the formation of a renewed scientific community focused on the collective dimension of health.

Discussion and conclusions

This PhD programme represents a cutting-edge training and research model, effectively responding to the threats posed by emerging infectious diseases. Based on extensive collaboration involving 14 universities and 9 research entities, it significantly contributes to the health system’s response capacity at national and international levels through multidisciplinary research.

T04 PATOGENESI E MECCANISMI DI VIRULENZA MICROBICI

24 - THE STREPTOCOCCUS AGALACTIAE PBSP PROTEIN IS INVOLVED IN A REGULATORY NETWORK CONNECTING THE SAER/S AND COVR/S TWO-COMPONENT SYSTEMS

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The Streptococcus agalactiae PbsP protein is involved in a regulatory network

connecting the SaeR/S and CovR/S two-component systems

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Introduction: PbsP is an important cell-wall adhesin that is under positive transcriptional control by the SaeR/S two component system (TCS) in the human pathogen Streptococcus agalactiae, also known as group B streptococcus or GBS. While SaeRS target genes are limited in number, a second TCS known as CovR/S regulates more than 100 genes, many of which encode for virulence factors. Among these, cyl operon genes encode for enzymes involved in the production of the hemolytic polyenic pigment of GBS. To evidence the possible presence of connections between these regulatory systems, we analyzed the phenotype of GBS strains with targeted mutations in SaeRS or PbsP.

Materials and Methods: We used the clonal complex 17 (CC17) BM110 strain to create a set of mutants in the SaeR/S TCS, including aconstitutively histidine kinase-actived (HKA) strain with and without deletions in pbsP or in bvaP, another SaeRS-controlled gene (HKA/BM110, HKA_bvaP/BM110 and HKA_pbsP/BM110) as well as CovR/S mutants. The mutants were evaluated for colony morphology and expression of the hemolytic pigment. Gene expression was measured by quantitative Real-Time-PCR (qRT-PCR) and transcriptome analysis.

Results: Constitutive activation of SaeRS (HKA/BM110) produced hyper-hemolytic colonies, compared to the isogenic wild-type (WT) strain, due to increased expression of cyl genes. Surprisingly, once pbsP, but not bvaP, was deleted in the HKA/BM110 background, increased hemolysis was abrogated, suggesting a specific contribution of PbsP to the regulation of CovRS-controlled genes. Transcriptome analysis confirmed the involvement of PbsP in the regulation of several virulence factors under the control of the CovR/S TCS.

Discussion and Conclusions: TCSs represents highly sophisticated systems that allow bacteria to modify virulence factors expression in response to different stimuli. In this study, we suggest that the GBS PbsP adhesin is involved in regulating the transcriptional expression of genes under CovR/S

control, thereby connecting this TCS with SaeRS. Whether PbsP acts directly or indirectly on Cov/RS is under investigation. These data may be useful to develop new anti-microbial strategies aimed at disrupting the expression of virulence factors in GBS.

25 - SAER/S, A STREPTOCOCCUS AGALACTIAE TWO COMPONENT REGULATORY SYSTEM, IS INVOLVED IN BIOFILM ASSEMBLY OVER HUMAN FIBRINOGEN

Giuseppe Valerio De Gaetano *(1)* ***- Francesco Coppolino*** *(1)* ***- Alessia Berbiglia*** *(2)* ***- Luca Tavella*** *(2)* ***- Maria Romeo*** *(1)* ***- Germana Lentini*** *(1)* ***- Concetta Beninati*** *(1)*

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SaeR/S, a Streptococcus agalactiae two component regulatory system, is involved in biofilm assembly over human fibrinogen.

DE GAETANO GIUSEPPE VALERIO1, FRANCESCO COPPOLINO1, ALESSIA BERBIGLIA2, LUCA TAVELLA2, MARIA ROMEO1, GERMANA LENTINI1 AND CONCETTA BENINATI1,3

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Introduction: Two-component regulatory systems (TCSs) are major mechanisms by which bacteria sense and respond to environmental changes. Environmental conditions that activate TCSs are numerous and diverse and may involve the presence of molecules on the surface of host epithelial cell with which bacteria interact to establish commensal or pathogenic relationships. Fibrinogen is constitutively expressed by a number of epithelial cells, including differentiated intestinal cells, and can promote vaginal persistence. Streptococcus agalactiae or GBS, a pathobiont that is part of the normal microbiota of the gastrointestinal and genitourinary tracts, is one of the main causes of neonatal infections, including pneumonia, sepsis, and fatal meningitis. In this study, we describe the influence of the SaeR/S TCS on formation of biofilms over fibrinogen-coated surfaces.

Materials and Methods: Using the crystal violet staining method, we examined the clonal complex 23 (CC23) NEM316 prototypical strain for its ability to form biofilms on human fibrinogen (Fg). A saeR gene deletion mutant (ΔsaeR) was used to verify the SaeR/S regulatory system contribution to biofilm formation. We also performed structured-illumination fluorescence microscopy analysis for revealing biofilm biomass, thickness and roughness by comparing wild-type (WT) and ΔsaeR strains. The fibrinogen-dependent CC23 biofilm was further compared with that formed by the prototype CC17 BM110 strain.

Results: Time-dependent biofilm formation studies revealed the capacity of GBS to create more robust and abundant biofilms on Fg than on collagen type IV or on abiotic surfaces after 48 hours of growth. Fluorescence microscopy analysis showed an orderly and progressive biofilm assembly on Fg, revealing areas of densely associated streptococci. Inactivation of the response regulator SaeR was

associated with reduced biomass, relative to its isogenic WT strain, using both the colorimetric and the fluorescence microscopy assay.

Discussion and Conclusions: Contrarily to abiotic supports, Fg-coated surfaces strongly promote biofilm formation by GBS and may represent useful models to study interactions between GBS and the host, as demonstrated by the ability of both NEM316 and BM110 strains to grow as sessile aggregates in a dense matrix. Moreover, it was possible -in the present study- to identify a role of the SaeRS TCS in biofilm formation. Studies are underway to establish whether the SaeR/S system regulates the expression of proteins capable of interacting directly or indirectly with Fg. Our results may be useful to clarify novel mechanisms underlying interactions between GBS and the human host and to develop new molecular strategies to control GBS persistence in the vaginal tract.

36 - IN VITRO STUDY OF INTERACTIONS BETWEEN MYCOBACTERIUM AVIUM PARATUBERCULOSIS AND HERV-W DERIVED-PEPTIDES WITH HUMAN PANCREATIC ISLETS: IMPLICATIONS FOR TYPE 1 DIABETES PATHOGENESIS.

Marta Noli *(1)* ***- April Joy Vergara*** *(2)* ***- Leonardo Antonio Sechi*** *(1)* ***- Reza Yarani*** *(3)* ***- Flemming Pociot*** *(3)*

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In Vitro Study of Interactions between Mycobacterium avium paratuberculosis and HERV-W derived-peptides with Human Pancreatic Islets: Implications for Type 1 Diabetes Pathogenesis.

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Introduction: Type 1 diabetes is a chronic autoimmune disease resulting from the destruction of pancreatic βeta-cells. Previous studies have shown that individuals with T1D, particularly at the onset of the disease, exhibit elevated antibody titers against peptides derived from Mycobacterium avium paratuberculosis (MAP) and Human endogenous retrovirus-W (HERV-W). However, the role of MAP and HERV-W in the pathogenesis of T1D remains to be elucidated. Human pancreatic islet was used to analyze the potential involvement of these two factors in beta-cell destruction. This in vitro model allows us to analyze potential interactions with beta-cells and also with different cell populations within the Human pancreatic islet. Our study aims to evaluate and compare protein transcripts by analyzing the whole secretome, collected post-treatment for 24h. Although, this would facilitate identifying and quantifying the proteins present, thereby providing valuable insights into the proteomic responses of the islets to the treatments. Materials and methods: Two highly immunogenic peptides (MAP 3865C125-133 and HERV-Wenv93-108) were chosen and tested on healthy human islets from a post-mortem donor. Human islets were seeded with a density of 120-130 islets per well in 12-well plates and were stimulated for 24 hours with 100.000 ng/mL of MAP and HERV-W derived peptides. The same plate also included a negative control of islets maintained in culture without conditioning and a positive control provided by cytokine cocktails comprising IL-1β and IFNγ. The supernatant, potentially containing proteins and the EVs secreted by the islets in response to the treatments, was carefully collected and preserved for subsequent proteomic analysis. Results and Conclusions: After a 24-hour conditioning period, an apparent decrease in viability of the treated islets is observed. MAP and HERV-W-treated islets show surprisingly similar cell viability to that observed with cytokines. This suggests these peptides probably exert effects comparable to cytokines on islet viability. We also

documented the morphological characteristics of islets, where it is possible to appreciate the effects of the treatments that subject the islets to stress or disaggregation and death.

39 - DETECTION AND ANALYSIS OF MERKEL CELL POLYOMAVIRUS IN ANAL SPECIMENS.

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Detection and Analysis of Merkel Cell Polyomavirus in anal specimens.

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Introduction: To date, most of the studies of viral infections of the anal tract have focused on Human Papillomavirus (HPV), well known to be associated with anal cancer. Specifically, anal cancer is common in Human Immunodeficiency Virus (HIV)-positive individuals, presumably because of the immunocompromised state leading to reactivation of latent HPV. However, as anal carcinoma can develop in the absence of HPV infection, other risk factors should be examined. On the basis of their carcinogenic potential, Human Polyomaviruses (HPyVs) have been proposed to be involved. Therefore, the aim of this study was to describe the prevalence of JC (JCPyV), BK (BKPyV) and Merkel Cell Polyomavirus (MCPyV) in anal specimens from 150 individuals and to investigate coinfections with HPV and/or HIV in those with known status. Materials and Methods: MCPyV, JCPyV and BKPyV DNA was detected by quantitative polymerase chain reaction (qPCR), followed by amplification and sequencing of Non Coding Control Region (NCCR) and Viral Protein 1 (VP1). Results: Regarding HPyVs distribution, 59/150 specimens were positive for at least one HPyV. Notably, MCPyV DNA was found in 49, JCPyV DNA in 12 and BKPyV DNA in 3 out of 150 samples, with a mean viral load of 4.3x105,7.55x102 and 3.1x102 gEq/mL, respectively. When HPyVs coinfection patterns were examined, the most common combination was MCPyV and JCPyV. Among the 76 HPV-positive patients, MCPyV infection was found to be the most prevalent, followed by JCPyV and BKPyV. Specifically, coinfection rates of carcinogenic HPV with MCPyV were significantly higher compared to those with JCPyV and BKPyV. Moreover, among the HIV-positive individuals with HPV infection, 12/32 were also tested positive for MCPyV, 1/32 for BKPyV and none for JCPyV. An increased MCPyV viral load was reported in MCPyV/HPV/HIV coinfected patients compared to those coinfected with MCPyV/HPV and to MCPyV-positive individuals. A canonical NCCR was observed in all MCPyV-positive samples whereas an archetype structure with the occurrence of point mutations, was found in samples positive for JCPyV and BKPyV. VP1 sequencing showed a high degree of homology with the reference strains in all samples, although 15/49 MCPyV-positive samples revealed nucleotide changes. Discussion and Conclusions: Our findings provide new insights into the HPyVs' prevalence in the anal tract, highlighting MCPyV as the most commonly detected. However, further studies are needed to clarify HPyVs' role in the development of anal disease. Considering the high MCPyV prevalence in anal

swabs and its well defined oncogenic properties, showing a cancerogenic model similar to that of HPV, special attention should be given to this virus, as it could be involved in cell transformation in the anal tract.

42 - THE PROTECTIVE ROLE OF TYPE I INTERFERON PATHWAY ACTIVATION IN AN IN VITRO MODEL OF VULVOVAGINAL CANDIDIASIS

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The protective role of type I interferon pathway activation in an in vitro model of vulvovaginal candidiasis

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Introduction

Previous studies showed that the commensal Candida albicans (Ca) 4314 strain, in contrast to the vulvovaginal candidiasis-associated strain Ca1887, up-regulates the type I interferon pathway in an in vitro model of a human epidermoid carcinoma A431 cell line. Here, we investigate the potential protective effect of type I interferon pathway in reducing the fungal shedding and the pro-inflammatory milieu activation.

Materials and Methods

After coculturing A431 cells with Ca4314 and Ca1887 strains for 1.5 h, the expression of interferon regulatory factor 3 (IRF3) was analyzed by FACS. In addition, the capacity of Ca1887 and Ca4314 in inducing fungal shedding by colony forming units (CFU) was investigated. The anti-IFNAR neutralizing Ab was included as a control for the involvement of type I interferon pathway in fungal shedding. Moreover, the release of IL-6, IL-8, IL-1alpha, and IL-1beta in supernatants was assessed by ELISA. Finally, the release of lactate dehydrogenase (LDH) was evaluated.

Results

The FACS analysis showed higher expression of IRF3 in A431 cells co-incubated with Ca4314 compared to A431 incubated with Ca1887. The analysis of fungal shedding showed a significant increase of CFU in Ca1887 compared to Ca4314. Moreover, the anti-IFNAR Ab enhanced the shedding of Ca4314, whereas such effect could not be detected in Ca1887. The ELISA showed increased levels of pro-inflammatory cytokines IL-1alpha and IL-1beta, in supernatants obtained from Ca1887-A431 co-colture, whereas no difference in IL-6 and IL-8 release was observed. The LDH analysis showed

higher cell damage in A431 co-incubated with Ca1887 cells compared to A431 incubated with Ca4314.

Discussion and Conclusions

Our results demonstrate the ability of Ca4314 in activating the type I interferon axis, in turn inducing lower levels of fungal shedding and cell damage. Our data also demonstrate the involvement of type I interferon pathway in reducing the pro-inflammatory potential of vulvovaginal candidiasis-associated strain Ca1887. Since the exact role of C. albicans in inducing VVC is yet to be elucidated, our results suggest that the stimulation/modulation of the type I interferon pathway may reduce the pathogenic effect of the fungus. Taken together, our data point to type I interferon pathway axis activation as a potential target to reduce the inflammation caused by C. albicans during VVC.

52 - DYNAMICS OF NASOPHARYNGEAL MYCOBIOTA IN COVID-19 AFFECTED INDIVIDUALS

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Dynamics of Nasopharyngeal Mycobiota in COVID-19 Affected Individuals

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Introduction. The nasopharyngeal tract hosts a diverse microbial community vital for maintaining mucosal host homeostasis. Recent findings indicate that SARS-CoV-2 infection disrupts the human nasopharyngeal microbiome, potentially influencing disease severity. While bacterial community alterations are well-studied, the impact of SARS-CoV-2 on the mycobiota remains poorly understood. Investigating fungal diversity dynamics in COVID-19 patients can advance our comprehension of disease pathogenesis and optimize disease management strategies. Materials and Methods. Nasopharyngeal swabs were collected from 55 COVID-19 positive patients with different symptoms in three distinct waves in the Campania Region. Of these patients, 39 had mild symptoms, 6 with moderate symptoms, and 10 with severe symptoms. Total RNA extraction from the samples was performed using the fully automated ELITeInGenius system and the ELITeInGenius SP RNA cartridge. Assessment of nucleic acid quality and quantity was performed using the Qubit RNA HS Assay Kit, followed by reverse transcription using the SensiFAST™ cDNA Synthesis Kit. Sequencing procedures were conducted using the NextSeq 500 platform. Fungal taxonomy profiling was achieved by leveraging the comprehensive RefSeq fungal genome/protein database. Results. Our analysis unveiled significant fluctuations in fungal diversity correlated with both the timing of sample collection waves and the severity of COVID-19 disease. Notably, patients sampled during the second wave exhibited heightened richness of nasopharyngeal mycobiota species. Conversely, fungal diversity in the nasopharynx remained relatively stable during the initial and subsequent waves of sample collection. The diminished mycobiota diversity observed during the first and third waves may stem from stringent pandemic-related measures enforced during the pandemic's onset and subsequent high positivity rates in post-summer periods. Furthermore, the phylum Ascomycota and its subphylum Saccharomycotina, alongside the phylum Basidiomycota, were more prevalent in patients with severe symptoms compared to those with mild symptoms. Several species within these phyla are recognized for causing co-infections that exacerbate infectious disease symptoms. Discussion and Conclusions. Our study reveals significant alterations in nasopharyngeal mycobiota due to SARS-CoV-2 infection,

influencing disease severity. Mycobiota diversity varies based on severity and timing of sample collection waves. Basidiomycota and Ascomycota are more prevalent in severe cases. Fungal diversity peaks during the second wave of collection, reflecting dynamic disease impact. COVID-19 induces dysbiosis in the fungal microbiome, potentially contributing to disease pathogenesis.

55 - NASOPHARYNGEAL PHAGEOME DYNAMICS: IMPLICATIONS FOR DISEASE SEVERITY AND PATIENT AGE ACROSS THREE COVID-19 WAVES

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Nasopharyngeal Phageome Dynamics: Implications for Disease Severity and Patient Age Across Three COVID-19 Waves

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Introduction. The global COVID-19 pandemic, driven by the SARS-CoV-2 virus, has generated a wave of scientific investigations into the impact of the microbiota on disease severity, patient age, and the temporal dynamics of disease progression. Often overshadowed in microbiome investigations is the key role of bacteriophages. This study attempts to comprehensively elucidate phage profiles under the aforementioned triad of conditions. Material and metods. This investigation encompassed 55 individuals who tested positive for SARS-CoV-2 within the Campania region. Nasopharyngeal swabs were systematically obtained during three distinct SARS-CoV-2 epidemics in Italy: March-May 2020 (n=25); September-November 2020 (n=25); January-February 2021 (n=5). The cohort was stratified based on symptom severity, with 39 cases categorized as non-severe, 6 as moderate, and 10 as severe. Patients enrolled in the study spanned an age range from 8 to 91 years (refer to Table 1). RNA extraction was executed utilizing the ELITeInGenius system, followed by RNA sample sequencing on the NextSeq 500 platform. Subsequent analysis of phage abundances was conducted employing the HOME-BIO 15 software suite. Results. In the analysis of 55 nasopharyngeal swabs obtained from COVID-19 patients, a total of 6 distinct phage families were discerned. Siphoviridae emerged as the most prevalent family, closely trailed by Myoviridae. Investigation into the temporal dynamics across the three pandemic waves unveiled that Peduovirinae exhibited higher abundance during wave I compared to wave II, a pattern echoed by Autographiviridae and Microviridae. Correspondingly, the initial phase displayed elevated frequencies of Peduovirinae, Autographiviridae, and Microviridae relative to the subsequent period. Notably, these phage families were more prevalent in samples derived from patients manifesting severe symptoms in contrast to those exhibiting non-severe conditions. Conversely, Siphoviridae, Myoviridae, and Microviridae exhibited diminished abundance in asymptomatic patients compared to the severe cohort. Regarding age stratification, significant distinctions were discerned solely within 2 phage families: Autographiviridae and Siphoviridae, which

exhibited reduced prevalence in patients aged 41–59 years relative to all other age groups. Conclusion. Throughout the progression of SARS-CoV-2 infection, notable shifts occur in the nasopharyngeal tract's phageome composition. These discernible changes offer valuable insights that can guide the development of more effective strategies for managing COVID-19.

62 - THE SERINE-RICH REPEAT GLYCOPROTEIN SRR2 MEDIATES STREPTOCOCCUS AGALACTIAE INTERACTION WITH HOST FIBRONECTIN

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The serine-rich repeat glycoprotein Srr2 mediates Streptococcus agalactiae interaction with host fibronectin

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Introduction: Group B Streptococcus (GBS) is a commensal of healthy adults and a potent pathogen in pregnant women, newborns and elderly adults. GBS possess different virulence factors to colonize the host. Among these, several cell-wall anchored (CWA) proteins have been characterized for their ability to promote host colonization and invasions. Srr2 is a CWA protein that represents a virulence factor able to bind fibrinogen (Fbg) through a dock, lock and latch (DLL) mechanism and plasminogen (Plg). The DLL mechanism consists in rearrangements of the N2 and N3 subdomains of Srr2 upon ligand binding. In this study, we report a new interaction of Srr2 with the human multidomain glycoprotein fibronectin (Fn) through the DLL mechanism. Fn is involved in cell adhesion and migration and is highly present in almost all human tissues. Importantly, we demonstrate that Srr2 interaction with Fn promotes bacterial adhesion to cervical epithelial cells.

Materials and methods: We tested by ELISA assays the ability of wild-type (wt) and mutant GBS strains to bind Fn. We prepared the recombinant forms of the binding region (BR) of Srr2 and produced mutant forms to study the mechanism of Fn binding. We calculated via BIAcore the binding affinity between Srr2-BR and Fn. By western-blot, we identified the Fn domain bound by Srr2-BR. Finally, we conducted adhesion assays by infecting monolayers of human cervical cells (HeLa) with GBS wt or delta-srr2.

Results: We demonstrate that Srr2 strongly enhances Fn binding by GBS ST-17 BM110 strain. The GBS delta-codY strain overexpressing Srr2 bound Fn more than the wt strain. Conversely, deletion of srr2 strongly decreased GBS ability to bind Fn. The Fn domain responsible for recombinant Srr2-BR binding was identified at the level of the central cell-binding domain of the protein. Mutations in recombinant Srr2-BR that abolished Srr2 ability to bind Fn allowed us to demonstrate that Srr2 binding mechanism

with Fn is DLL. Finally, the concomitant presence of Srr2 on the bacterial surface and Fn in the culture medium increased GBS adhesion to cervical epithelial cells.

Discussion and Conclusions: Our data report a novel important role for Srr2 in GBS adhesion to Fn and demonstrate the importance of this interaction in promoting GBS adhesion to the host cells. In light of this, our results may be useful to develop new alternative strategies to control GBS infection and prevent colonization of epithelium.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases.

70 - SINGLE AMINO ACID SUBSTITUTION IN FIBRONECTIN BINDING PROTEIN A (FNBPA) OF S. AUREUS STRONGLY REDUCES CROSS-LINKING WITH FIBRIN BY BACTERIALLY-ACTIVATED FXIII

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Single amino acid substitution in Fibronectin Binding protein A (FnBPA) of S. aureus strongly reduces cross-linking with fibrin by bacterially-activated FXIII

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Introduction

Multi-drug resistant (MDR) Staphylococcus aureus is a significant danger to public health, prompting a critical need for research into novel drug targets and virulence factors. Von Willebrand factor binding protein (vWbp), initially identified as a bacterial receptor for the human von Willebrand factor (vWF), plays a crucial role in the pathogenicity of S. aureus, involving the binding and non-proteolytical activation of prothrombin, Factor XIII (FXIII) activation and, ultimately, fibrin cross-linking. In our prior work, we investigated the ability of bacterially-activated FXIII to cross-link an S. aureus adhesin, Fibronectin Binding protein A (FnBPA), to the fibrin network, resulting in complex heteropolymers formation. In this work, we continued to investigate this mechanism, producing a recombinant single amino acid mutant protein in the N1 subdomain of the A region in a residue identified as a major cross-linking site.

Materials and Methods

We prepared a recombinant mutant form of FnBPA N1 subdomain through a single amino acid substitution of Gln103 to Ala (Q103A) to investigate its role in the cross-linking to fibrin(ogen) mediated by bacterially-activated FXIII. Cross-linking of recombinant proteins to fibrin(ogen) was evaluated with Western immunoblotting (qualitative assay) and ELISA (quantitative assay).

Results

We proved that substituting Gln103 with Ala in the N1 subdomain of FnBPA the production of high molecular weight heteropolymers through the action of vWbp-activated FXIII is strongly reduced.

Discussion and Conclusions

The functional reactive Gln103 residue, previously identified as the main lysine acceptor residue within fibrinogen, is fundamental to drive the formation of high molecular weight heteropolymers with fibrin(ogen). Supporting this evidence, multiple alignments of the N-terminal sequence of various S. aureus cell-wall anchored proteins with FnBPA showed that none of them possess a reactive Gln103

residue, and in fact none is able to be cross-linked to fibrin(ogen) as previously published. These discoveries highlight a distinctive pathogenic mechanism, wherein the transglutaminase activity of bacterially-activated FXIII could play a relevant role in the persistence of S. aureus inside the host.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases.

72 - INVESTIGATING PYROPTOTIC PATHWAYS IN A MOUSE MODEL OF MENINGOCOCCAL MENINGITIS: INSIGHTS INTO THE ROLE OF HRPA/HRPB TWO-PARTNER SECRETION SYSTEM.

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Investigating pyroptotic pathways in a mouse model of meningococcal meningitis: insights into the role of HrpA/HrpB Two-Partner Secretion System.

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Introduction. Two-partner secretion (TPS) systems are secretion pathways used by many pathogenic Gram-negative bacteria for the release of large proteins involved in the interaction between bacteria and their hosts, and in inter-bacterial competition or cooperation. These systems rely on an exoprotein harboring an N-terminal secretion domain (TpsA) and a channel-forming β-barrel activator/transporter protein (TpsB) required for transport of the exoprotein across the outer membrane. The genome of Neisseria meningitidis strains may encode up to three different TPS systems. In particular, HrpA/HrpB TPS has emerged as a key player in establish meningococcal meningitis. The aim of the present study was to evaluate the role of HrpA/HrpB TPS in establishing meningococcal meningitis and activating pyroptotic pathways in an experimental murine model based on intracisternal infection of female BALB/c adult mice. Materials and Methods. We employed the serogroup C reference strain 93/4286 and an isogenic mutant 93/4286ΩhrpB, which lacks the expression of HrpB transporter. In in vivo experiments, we assessed the 50% lethal dose (LD50) of these strains and examined their replication in the mouse brain and peripheral organs (liver and spleen). To examine the ability of 93/4286

reference strain and 93/4286ΩhrpB isogenic mutant to activate the pyroptotic pathway, Western blot analyses were conducted focusing on the principal markers of the pyroptotic process (caspase-1, caspase-11, GASDMD, and caspase-7) and on the main proinflammatory cytokines involved in this pathway (IL-1 beta, TNF-alfa) in the murine brain. Results. Survival experiments confirmed the role of HrpA/HrpB TPS in the invasive meningococcal disease. In fact, the ability of the hrpB mutant to replicate in mice brain and spread systemically was severly impaired. Furthermore, Western blot analysis of murine brain samples during the meningococcal infection demonstrated that N. meningitidis activated canonical and non-canonical inflammasome pyroptosis pathways while the activation of caspase-11, caspase-1, and GASDMD was markedly reduced in the mice infected with hrpB-mutant, Furthermore, the increased amount of IL-1 beta, an important end point of pyroptosis, occurred only in the brains of mice infected with the wild type strain 93/4286 and not in those infected with 93/4286ΩhrpB. Discussion and Conclusions. These results, obteined from an experimental murine model, suggest that the HrpA/HrpB TPS plays a crucial role in the induction of pyroptosis and suggest a pivotal involvement of pyroptosis in invasive meningococcal disease, hypothesing the use of pyroptosis inhibitors in the adjuvant therapy of the disease.

75 - INHIBITION OF SARS-COV-2 CELL ENTRY BY HEPARAN SULFATE AND ENOXAPARIN: A COMBINED IN SILICO AND IN VITRO STUDY

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Inhibition of SARS-CoV-2 cell entry by heparan sulfate and enoxaparin: a combined in silico and in vitro study

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Introduction: The attachment and entry of SARS-CoV-2 inside mammalian cells are primarily mediated by human angiotensin-converting enzyme 2 (ACE2) and its interaction with the spike protein. However, it's widely recognized that the spike protein also interacts with other molecules such as glycosaminoglycans (GAGs), including heparan sulfate (HS) and Enoxaparin (EX), which are linear, anionically charged polysaccharides with known biological activities. These polysaccharides function by binding to the spike protein, thereby obstructing its interaction with ACE2 receptors. Materials and Methods: This study aimed to assess a model to confirm the activity of these GAGs in Pseudovirus SARS-CoV-2. This was achieved by combining in silico modeling with in vitro determination using BacMam technology. Results: The findings demonstrated the concentration-dependent antiviral activity of HS and EX, providing a resolution to conflicting results observed in recent studies on SARS-CoV-2 cellular entry. In conclusion, these results underscore the potential of HS and EX in combating SARS-CoV-2, shedding light on intervention strategies targeting the virus's cell entry mechanisms. By establishing a safe and effective in vitro model, this study offers valuable insights into developing therapies aimed at mitigating the variability of SARS-CoV-2 and preventing viral entry into host cells. Discussion and Conclusions: This research opens new avenues for exploring the therapeutic potential of GAGs and their derivatives in combating COVID-19.

81 - ADVANCED 2D AND 3D IN VITRO MODELS OF NEURAL CELLS TO TEST ANTIVIRAL DRUGS FOR CONGENITAL CYTOMEGALOVIRUS INFECTION

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Advanced 2D and 3D in vitro models of neural cells to test antiviral drugs for congenital cytomegalovirus infection

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Introduction: Congenital Cytomegalovirus infection (cCMV) is a significant public health burden and there are currently no approved antiviral drugs to treat this disease. Studies of HCMV pathogenesis and drug testing in human fibroblasts do not accurately replicate the target cells affected in cCMV, i.e. the neural stem cells (NSCs) of the developing cerebral cortex. The aim of this study was to develop a new in vitro platform for antiviral drug discovery that utilizes more disease-relevant cell types affected during cCMV.

Methods: We used a platform of 2D human embryonic stem cell (hESC)-derived neural stem cells (NSCs), a neuroepithelial stem (NES) cell line derived from prenatal human samples, and 3D hESC-brain organoids. NSCs and NES cells were infected with HCMV and the toxicity and efficacy of different antiviral drugs, i.e. ganciclovir (GCV), letermovir (LTV), nitazoxanide (NTZ) and ozonide OZ418, were tested to determine the selectivity index (SI) for each compound. Relative quantification of viral DNA by qPCR was used to confirm inhibition of viral replication. The neuroprotective effects of the drugs were investigated by evaluating the modulation of the expression of various neurogenesis markers by qPCR. Brain organoids were obtained from hESCs and infected with HCMV TB40-pp65-YFP to analyze the neuropathogenesis of the virus in a model that mimics the architecture of the developing brain. Thirty-day-old organoids were infected and treated for 3 weeks with the same antiviral drugs previously tested. Fluorescence signals from infected and uninfected organoids were measured at different time points and viral protein expression was analyzed by Western blot after 21 days.

Results: GCV, LTV, NTZ and OZ418 showed favorable SI in infected NSCs and NES cells. In addition, a reduction in viral DNA synthesis confirmed the efficacy of the drugs in blocking viral replication. HCMV

infection led to deregulation of key neural progenitor cell markers such as DCX, PAX6, SOX2 and PPAR-γ, which was partially reversed by antiviral treatment. In parallel, HCMV-infected organoids showed altered expression, localization, and organization of neural-specific markers such as DCX, NESTIN and TUBB3. Infected organoids showed a marked reduction in fluorescence signal for all drugs. Treatment with NTZ resulted in impaired growth of both infected and mock-infected organoids. All other drugs tested did not affect organoid morphology and resulted in a significant reduction of viral proteins after 21 dpi.

Conclusions: NSCs, NES cells and brain organoids may represent useful models of disease-relevant cell types to investigate both the antiviral and neuroprotective potential of therapeutic candidates against cCMV.

84 - ELISABETTA

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Establishment of an in vitro model to study co-infection of vaginal epithelium by Candida albicans and Herpes Simplex Virus 2

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Background and aim. Most pathogens enter the human body by breaching the mucosal barriers, thus establishing a successful infection. Candida albicans (Ca) and Herpes Simplex Virus 2 (HSV-2) are among the most common pathogens of the genital tract. The present study aims to develop an in vitro model that, by closely mimicking human genital mucosa, will allow to dissect the fine host-pathogens interactions during mono- and polymicrobial infections.

Materials and Methods. The A-431 vaginal cell line was used to set up cultures as: i) monolayers, produced on 96 or 24 multi-well plates, after 24 h of incubation in DMEM with 10% FBS; ii) reconstituted vaginal epithelium (RVE), established after 5-6 days of incubation under that same culture conditions, with medium renewal at day 3; iii) vaginal cells cultured on inert scaffolds. The cells, exposed or not to an artificial vaginal fluid (AVF), were infected with Ca and/or HSV-2, at the multiplicity of infection (MOI) of 10:5:1. Epithelial viability and cytokine production were assessed by LDH release and ELISA assays, respectively; Ca and viral load were also evaluated, at different time points and conditions, by CFU assay and PCR-based DNA quantification, respectively.

Results. The epithelial cells, no matter whether assessed as monolayer or as RVE, were partially affected by the addition of the AVF. Differently, Ca exposure to AVF consistently enhanced its survival and growth ability. The epithelial cell viability was significantly impaired upon infection with Ca, while HSV-2 caused little or no effects; the combination of the two pathogens resulted in slightly additive effects in terms of cell damage. To date, the monolayer proved to be the best condition to assess cytokine production in response to a single or double infection. In particular, epithelial cells produced

IL-8 following either HSV-2 or Ca infection, while the double infection did not further enhance such chemokine production. Differently, IL-1beta was released by Ca infected cells only; HSV-2 had no effects, either when added alone or in combination with Ca. Initial experiments performed by culturing the A-431 cells onto inert scaffolds indicated the ability of the cells to colonize such tridimensional matrix. Histological analysis of such cultures and viral load quantification are in progress.

Discussion and Conclusions. The A-431 epithelial cells are susceptible to both HSV-2 and Ca infection; they respond to the pathogens to a different extent, also depending upon the presence of the artificial vaginal fluid. These results lay the foundation for the development of an in vitro prototype that, by closely resembling the in vivo mucosal environment, will allow to highlight both single and cumulative microbial impact on host vaginal mucosa.

101 - MICROBIOLOGICAL EVALUATION OF PERILESIONAL SKIN AND PUNCH BIOPSIES FROM HIDRADENITIS SUPPURATIVA PATIENTS

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Microbiological evaluation of perilesional skin and punch biopsies from hidradenitis suppurativa patients

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Introduction: Hidradenitis suppurativa (HS) is an inflammatory skin disease characterized by small, painful lumps formed under the skin, but its exact etiology is not well understood. In recent years, researchers have been investigating the potential role of bacteria in HS, but conflicting results have accumulated over time. Thus, it still remains uncertain whether bacteria are the cause of HS or merely a consequence of the ongoing inflammatory process. Therefore, further research achieving standardization in microbiological methods, minimizing variability, and ensuring the precision of skin microbiome data would be crucial for better understanding of the microbial role in HS etiology and pathogenesis.

Materials and Methods: A group of 14 HS patients with Hurley stages II and III (i.e., presenting recurrent abscesses and sinus tract formation), were included for microbiological analyses, prior to antibiotic treatment. To this aim, superficial cutaneous swab samples and punch biopsy samples from the lesions of each patient were collected and the aerobic microflora cultured and quantitatively measured from each sample under standard laboratory conditions. In addition, the pH of the lesions at the level of draining tunnels and the perilesional skin areas were measured. A non-invasive Moleculight fluorescence device, reported to detect bacteria in wounds containing elevated bacterial loads, was also used to evaluate the presence or absence of bacteria in the lesions.

Results: Microbiological culture analyses demonstrated that almost all samples were polymicrobial containing at least 2 or 3 different bacterial species. Bacterial load of swab samples (CFU/gr) was significantly higher than biopsy samples ($P<0.05$). Most of the bacteria isolated were part of the normal microbial flora of the skin. Staphylococcus spp was isolated at higher frequency (from 9/14 biopsies and from all the swab samples) followed by Corynebacterium spp (from 6/14 biopsies and from 8/14 swabs). Proteus mirabilis, a component not usually part of the skin microflora was isolated

from 3 biopsy samples, but from only one swab sample. The comparison between culture and Moleculight fluorescence device results is in progress.

Discussion: The preliminary results obtained from our ongoing study revealed a dominant colonization of HS lesion samples with normal skin commensal flora with few exceptions. This observation is in agreement with previous work, suggesting that commensal bacteria may elicit inflammatory responses in genetically susceptible individuals contributing to the pathogenesis of the disease. Microbiome analysis of profound HS lesions (biopsy samples) compared to the superficial (swab) ones may give further insights to the role of bacteria in the etio-pathogenesis of the disease.

103 - EXPLORING THE ROLE OF THE ESX-2 SECRETION SYSTEM IN THE INTRACELLULAR GROWTH OF MYCOBACTERIUM TUBERCULOSIS

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Exploring the role of the ESX-2 Secretion System in the intracellular growth of Mycobacterium tuberculosis

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Introduction

Mycobacterium tuberculosis (Mtb), the causative agent of human tuberculosis, has evolved successful strategies to invade/replicate within phagocytic cells, thus eluding host immunity. The key role of mycobacteria-specific secreted proteins in modulating the intracellular growth/survival of Mtb is well known. Particularly, effector molecules exported via selected type VII/ESX secretion systems (ESX-1, ESX-3, and ESX-5) have been found as major determinants of Mtb-host cell interplay. In contrast, the ESX-2 secretion system and its secreted substrates remain poorly characterized, and their biological role is still unclear. To characterize the ESX-2 secretion system and investigate its impact in Mtb-macrophage interactions, two H37Rv Mtb mutant strains, inactivated for the ESX-2-related EccC2 ATP-ase (MtbeccC2KO), or the ESX-2-encoded substrate EsxC (MtbdeltaesxC), were constructed. Complemented strains, expressing a functional ESX-2 system, were also obtained.

Materials and Methods

The MtbeccC2KO and MtbdeltaesxC mutants were constructed via the recombineering strategy, allowing the replacement of the wild-type copy of the target gene with an inactivated copy by allelic exchange mediated by the mycobacteriophage Che9c-derived gp60/gp61 recombinases. Complemented strains were obtained by integrating the I106w cosmid, carrying the entire esx-2 gene cluster, into the chromosomal attB site of each mutant. The growth kinetics of mutants, wild-type and complemented strains were evaluated both in liquid media (e.g. Middlebrook 7H9) and in human macrophages. PMA-differentiated THP-1 human macrophages were infected at two different MOI (10:1 or 25:1, bacteria:cell). Infected monolayers were lysed immediately after phagocytosis (1.5 h) and 2, 4, and 6 days following infection, after which the number of viable intracellular bacteria were determined.

Results

ESX-2 mutants showed similar growth kinetics in growth ability as compared to control strains in Middlebrook 7H9 medium. When tested in the THP-1 ex vivo model, ESX-2 mutants displayed similar phagocytosis efficiency as compared to the wild-type and complemented stains, but a reduced intracellular replication ability relative to the wild-type strain over a 6-day period. However, such impaired replication was less pronounced as compared with that displayed the by an ESX-1 deletion mutant, included as additional control. The complementation with the intact esx-2 locus almost completely restored the intracellular growth properties.

Discussion and Conclusions

Data obtained indicate that ESX-2 and its secreted substrates are not required for Mtb growth in nutrient rich media neither for efficient bacteria engulfment by macrophages, but might contribute to the optimal intracellular growth of Mtb.

104 - GENOMIC AND PHENOTYPIC CHARACTERIZATION OF A PRESUMPTIVE HYPERVIRULENCE CLOSTRIDIOIDES DIFFICILE RIBOTYPE 027 STRAIN

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Genomic and phenotypic characterization of a presumptive hypervirulence Clostridioides difficile ribotype 027 strain

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Introduction

Clostridioides difficile (C. difficile) is an anaerobic, spore-forming, gram-positive bacterium that produces toxins. It is a ubiquitous organism and forms part of the normal bacterial flora in the gastrointestinal tract of both animals and humans. In nature, there are hypervirulent strains, such as ribotype 027, which overexpress toxins tcdA and tcdB and can also produce the binary toxin cdt (C. difficile transferase).

Materials and Methods

A sample of diarrheal feces, deemed suitable for C. difficile research according to the Bristol scale, was analyzed. This sample was subjected to enzyme immunoassays (EIA) (Liason, DiaSorin Inc. Italia) to detect Glutamate dehydrogenase (GDH), tcdA, and tcdB. Additionally, nucleic acid amplification tests (NAAT) Cepheid (Sunnyvale, CA, USA) and Seegene Inc. (Korea Exhange, KRX), based on real-time PCR, were conducted to search for genes tcdB and cdt, targeting both toxin and DNA. Furthermore, the sample was individually seeded on selective chromogenic medium CDIF bioMérieux (Marcy-l'Etoile, Francia), recognized as the gold standard for detecting toxigenic strains. Subsequently, the cytotoxic power was evaluated in vitro on VERO cells. Additionally, whole-genome NGS Illumina Inc. (San Diego CA, USA) analysis was performed on both samples.

Results

The stool sample was positive for GDH in the EIA test but negative for toxins tcdA and tcdB; however, it was positive in the NAAT tests for the presence of genes tcdB and cdt, indicating its classification presumptive ribotype 027. The sample grew on CDIF medium, but it did not exhibit cytotoxicity in the VERO cell. Furthermore, whole-genome sequencing analysis revealed the sample having approximately a 10% difference in the tcdB gene compared to the wild type gene.

Discussion and Conclusions

The ribotype 027 of C. difficile presents a deletion of the tcdA and tcdB genes, which leads to overexpression of the genes and, in addition, the expression of the cdt gene; in our study, we analyzed a case of ribotype 027 that did not exhibit cytotoxicity in VERO cell, which was also explained by whole-genome sequencing, where sample had a sequence homology of less than 90% in toxins tcdB.

105 - THE EXPRESSION OF M PROTEIN IN THE BIOFILM OF STREPTOCOCCUS PYOGENES IN INVASIVE AND NON-INVASIVE STRAINS

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The expression of M protein in the biofilm of Streptococcus pyogenes in invasive and non-invasive strains.

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Introduction

Streptococcus pyogenes causes a wide range of pathological manifestations, ranging from superficial infections to potentially lethal invasive diseases. In S. pyogenes, the M protein, encoded by the emm gene, plays an important role in biofilm formation and its virulence. The aim of this study was to characterize the in vitro biofilm formation capacity of S. pyogenes in patients with invasive and non-invasive infection based on the type of M protein. To evaluate the biofilm formation in vitro assays can be set up to assess biomass, using stains such as Crystal Violet (CV) and metabolic dyes.

Materials and Method

Sixteen strains of S. pyogenes were analyzed: 8 causing invasive infections, 8 from colonized districts (Table 1). The samples were collected at the O.U. Microbiology of the Great Romagna Hub Laboratory, Cesena, Italy. The whole genome was sequenced using the Illumina Inc. platform (San Diego, U.S.A). Subsequently, they were cultured in vitro using Mueller-Hinton Broth+3% LHB agar (Beckman Coulter, Brea U.S.A.) to assess biofilm growth. A volume of 100µl of cell suspension at 1.5x107 CFU/ml was incubated at 37°C in flat-bottom micro wells (T30096, SPL) and evaluated at 2h, 24h, and 48h, using a spectrophotometer with CV (595nm) and Alamar Blue metabolic dye (570nm).

Result

Whole genome sequencing revealed M proteins belonging to different clusters, with a higher production of biofilm in the invasive strains 1 and 2 (relatives) with M protein 100.7, and in strain 5 with M protein 89. No significant growth was observed in the non-invasive strains, and the isolates with M protein 89 exhibited different biofilm production in invasive versus non-invasive strains.

Discussion and Conclusions

The incidence of Group A Streptococcus (GAS) related infections in recent years has led to increased surveillance of associated infections. Biofilm production doesn't seem to be correlated with a specific M protein; therefore, further genomic analyses could be useful in studying factors that may increase its virulence.

	Material	**Pathology**	**Protein M**	**CV (OD ± SD)**
1	Blood cultures	Bacteremia	100.7	0,253±0.221
2	Blood cultures	Bacteremia and endocarditis	100.7	0,283±0.170
3	Blood cultures	Bacteremia	1	0,104±0.014
4	Blood cultures	Bacteremia	1	0,113±0.024
5	Ulcer vascular exudate	Ulcer	89	0,279±0.233
6	Blood cultures	Bacteremia and respiratory failure	8.4	0,144±0.060
7	Blood cultures	Severe hypotensive shock	1	0,127±0.052
8	Blood cultures	Bacteremia	1	0,161±0.109
9	Oropharyngeal swab	Pharyngitis	1	0,130±0.046
10	Oropharyngeal swab	Pharyngitis	81	0,138±0.060
11	Oropharyngeal swab	Pharyngitis	12	0,127±0.037
12	Oropharyngeal swab	Pharyngitis	89	0,184±0.082
13	Oropharyngeal swab	Pharyngitis	89	0,186±0.090
14	Oropharyngeal swab	Tonsillitis	87	0,175±0.065
15	Oropharyngeal swab	Pharyngitis	89	0,139±0.038
16	Oropharyngeal swab	Pharyngitis	77	0,150±0.072

114 - COINFECTION OF DERMAL FIBROBLASTS BY HUMAN CYTOMEGALOVIRUS AND HUMAN HERPESVIRUS 6 CAN BOOST THE EXPRESSION OF FIBROSIS-ASSOCIATED MICRORNAS

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Topic: Patogenesi e meccanismi di virulenza microbici

Coinfection of dermal fibroblasts by Human Cytomegalovirus and Human Herpesvirus 6 can boost the expression of fibrosis-associated microRNAs

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Introduction: Human cytomegalovirus (HCMV) and Human herpesvirus type-6A (HHV-6A) have been reportedly suggested as triggers of the onset and/or progression of many autoimmune diseases, including Systemic Sclerosis (SSc), a severe autoimmune disease with still unclarified etiology, causing progressive fibrosis of skin and internal organs. Reactivation of such viruses and specific antiviral immune responses have been detected in SSc patients, and infection by HCMV or HHV-6A was shown to induce the expression of fibrosis-associated transcriptional factors and miRNAs in human dermal fibroblasts. However, it is unlikely that such viruses have separated effects on infected cells since both viruses are ubiquitously present in the human population and can mutually boost each other. Consistently, we recently reported that the simultaneous presence of HCMV and HHV-6 induced a higher expression of fibrosis-associated factors associated, compared to what observed in single infected cells. Based on these observations, this study aimed to investigate in primary human dermal fibroblasts (the primary targets of SSc), the effect HCMV/HHV-6A coinfection, focusing on the expression of miRNAs associated with pro-fibrotic pathway.

Materials and Methods: Human primary dermal fibroblasts were infected in vitro with cell-free inocula of HCMV (T40E) and HHV-6A (U1102), and samples were collected at different times post infection (0, 1, 2, 4, 7 and 10 d.p.i.). Total nucleic acids were extracted from collected cells and analyzed by virus-specific real-time quantitative PCR (qPCR), and by qPCR microarrays simultaneously detecting and quantifying 84 human microRNAs associated with cell fibrosis.

Results: The results evidenced the simultaneous coinfection of fibroblasts with HCMV and HHV-6A significantly enhanced the replication of both viruses, which was in turn accompanied by increased induction of fibrosis-miRNAs in coinfected cells compared to single-infected cells.

Discussion and Conclusions: The collected data support the hypothesis that HCMV and HHV-6 can enhance each other and may cooperate at inducing enhanced miRNA driven fibrosis in coinfected individuals. These data also suggest that the analysis of virus-induced miRNAs may represent a novel diagnostic or prognostic biomarker for SSc and its clinical treatment.

123 - SELECTIVE HISTONE DEACETYLASE 6 (HDAC6) INHIBITORS MODULATE THE VIRULENCE AND BIOFILM FORMATION OF PSEUDOMONAS AERUGINOSA.

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Selective histone deacetylase 6 (HDAC6) inhibitors modulate the virulence and biofilm formation of Pseudomonas aeruginosa.

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1. Introduction: Bacterial infections represent a significant public health concern, particularly due to the rise of multidrug-resistant strains. In recent times, breakthroughs in the epigenetics field have shed light on its involvement in microbial infections, prompting substantial research initiatives in this area. Reversible acetylation, a protein post-translational modification, stands out for its role in responding to environmental cues and maintaining cellular balance. The histone deacetylase 6 (HDAC6) enzyme has been demonstrated to reduce the bacterial load and the inflammatory markers in a murine model of chronic Pseudomonas aeruginosa infection, showing a pivotal role in the intricate molecular processes regulating bacterial clearance and survival. Here, we unveil for the first time the ability of a potent HDAC6 inhibitor to interfere with the biofilm formation and modulate virulence factors in P. aeruginosa, marking a significant advancement in our understanding of bacterial pathogenesis. 2. Materials and methods: The newly synthesized compound F2F-2020202, showing high potency on HDAC6 and high isoform selectivity over HDAC1, was tested on P. aeruginosa ATCC 27853 and P. aeruginosa PAO1 (ATCC BAA-47-B1). The bacteria antimicrobial susceptibility test, the motility and biofilm inhibition assays, and the pyocyanin and rhamnolipid dosage were performed. Expression levels of several quorum sensing (QS) genes were analyzed by quantitative real-time polymerase chain reaction. 3. Results: We demonstrated that compound F2F-2020202 altered the normally balanced production of the virulence factors pyocyanin and rhamnolipids in both strains of P. aeruginosa. This imbalance was also accompanied by a reduction of the swarming motility and a disrupted biofilm structure and organization. Additionally, we highlighted the potential involvement of F2F-2020202 in the QS mechanism inducing a differential regulation of specific genes, such as RhlI, phAz1, and qsrO. 4. Discussion and conclusions: Our study paves the way for future in-depth investigation to allow the complete elucidation of the molecular mechanisms underlying F2F-2020202 activity. Pyocyanin and rhamnolipids are two crucial virulence factors of P. aeruginosa. In pulmonary infections involving P. aeruginosa, they play a central role in biofilm formation and pathogenicity. Therefore, the significant reduction in pyocyanin and rhamnolipid production and the subsequent biofilm disruption induced by the compound F2F-2020202 should lead to a recovered protective immune response, thereby preventing the bacterium's persistence in the lung.

151 - IMPACT OF IN VITRO SARS-COV-2 VIRAL INFECTION ON BREAST CANCER CELLS

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Impact of in vitro SARS-CoV-2 viral infection on breast cancer cells

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Background: Coronavirus disease 19 (COVID-19) pandemic, determined by SARS-CoV-2, had severe repercussions on breast cancer patients. Increasing evidence indicates that SARS-CoV-2 infection may directly impact on breast cancer biology but the effects of SARS-CoV-2 on breast tumor cell are still unknown.

Materials and Methods: In this study, we analyzed the molecular events occurring in MCF7, MDAMB231 and HCC1937 breast cancer cell lines, representative of luminal A, basal B/claudin low and basal A subtype, respectively, upon SARS-CoV-2 infection. Viral replication was monitored over time and gene expression profile was conducted.

Results: We found that MCF7 cells resulted the most permissive to viral replication. Treatment of MCF7 cell line with tamoxifen reduced SARS-CoV-2 replication rate, suggesting a possible involvement of the estrogen receptor (ER) in sustaining virus replication in malignant cells. Interestingly, a metagene based on genes found to be up-modulated in all the three cell lines by SARS-CoV-2 identified a subgroup of pre-menopausal Luminal A breast cancer patients experiencing poor prognosis.

Discussion and Conclusions: As SARS-CoV-2 still spreads among the population, it is essential to better understand the direct impact of SARS-CoV-2 infection on breast cancer, particularly in pre-

menopausal patients diagnosed with luminal A subtype, and to assess the long-term impact of Covid-19 on breast cancer outcomes.

152 - EXPRESSION OF HUMAN ENDOGENOUS RETROVIRUSES (HERV) IN COLON CANCER PATIENTS

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Expression of Human Endogenous Retroviruses (HERV) in colon cancer patients

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Introduction: Human Endogenous Retroviruses (HERV) constitute 8% of human genome where they are integrated, and usually they are not transcribed. Despite their inactivation, many retroviral sequences present intact ORF-able to produce retroviral transcripts and/or proteins, which can be detected in a variety of human cancers. Research regarding HERV gene expression in colon cancer, the third most common tumor worldwide, is needed.

Materials and Methods: A metanalysis of three studies, comprising 177 Italian and Tunisian advanced-stage colon cancer patients, with a median age of 73 years old, was performed. HERV-H, -K, -P env gene, and HERV-K pol gene expression level was analysed in tumor and healthy tissues, and, when possible, in peripheral blood. Associations among clinical characteristics and HERV gene expression levels were analysed. The presence of HERV transcripts was evaluated in plasmatic Extracellular Vesicles (EV), isolated from 68 patients. HERV-K Env protein expression was evaluated in tumor and healthy tissue of 23 patients by Western Blot.

Results: HERV gene expression level was similar among biological samples. Significant higher expression of HERV-H and -P env was observed in tumor tissues arising from left colon and from right colon, respectively. In tumor tissues, HERV-H and -P env genes were more expressed in patients under 73, and over 73 years old respectively and higher levels of HERV-H and -K env genes were observed in the late stage compared to the early stage of the tumor. HERV-H, -K, -P env, and -K pol genes were

expressed in 32%, 38%, 15%, and 12% of EV respectively. HERV-K Env protein was more expressed in tumor than healthy tissues.

Discussion and Conclusions: HERV env gene expression seems to represent a specific signature in tumor tissue, based on tumor location, stage and patients' age. HERV sequences in plasmatic EV might be transferred from one cell to another, favouring cellular transforming mechanisms. Expression of HERV Env protein in tumor tissue may induce cell to cell fusion, contributing to cancer developing.

173 - SANDFLY FEVER NAPLES VIRUS TRIGGERS THE UNFOLDED PROTEIN RESPONSE PATHWAY IN AN IN VITRO INFECTION MODEL

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Sandfly Fever Naples Virus triggers the unfolded protein response pathway in an in vitro infection model

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Introduction. Sandfly viruses are RNA enveloped viruses belonging to the Phlebovirus genus. They are endemic to several world regions but recently have been gaining attention due to their spread to new areas and the ongoing isolation of new species. Indeed, Sandfly Fever Naples (SFNV), Toscana and Sicilian viruses, the most common members, have registered an increasing number of infections in humans, some of them with a poor outcome. The unfolded protein response (UPR) is an adaptive cellular response triggered when nascent and unfolded polypeptides exceed the ER folding capacity, under a plethora of stress conditions. The UPR activation can be considered a double-edge sword playing pro-viral or anti-viral effect depending on the virus and the infection stage. Here we evaluated the role of UPR in an in vitro infection cellular model of SFNV, analyzing the expression of the main UPR effectors. Materials and Methods. The human adenocarcinoma cell line (A549) was infected with SFNV and a time-course assay (from 24 hours post-infection to 120 hours post-infection) was carried out to evaluate the effect of viral replication on the cellular UPR. Total RNA and proteins were extracted at different time points to perform quantitative Real Time PCR (q-RT-PCR) and Western Blot analysis, respectively. Results. The genic expression study revealed a marked and significantly upregulation of the UPR master regulator GRP78 and the three branches’ (PERK, ATF-6 and IRE-1) transcripts at later stages of infections (72 – 120 hours post-infection) compared to the uninfected control. As well, we found the mRNA levels of their main downstream effectors (CHOP, ATF-4 and spliced XBP-1) upregulated. The protein levels agreed with the transcripts’ dosages as indicated by Western Blot analysis. Discussion and Conclusions. To the best of our knowledge, these is the first evidence reporting the involvement of UPR in the replication and pathogenesis of SFNV. Further studies are required to address the role of each of the three branches downstream effectors signaling pathways.

186 - EVOLUTIONARY DYNAMICS AND COMPARATIVE ANALYSIS OF ACINETOBACTER BAUMANNII CLINICAL ISOLATES: INSIGHTS FROM GENOMIC AND PHENOTYPIC APPROACHES

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Evolutionary dynamics and comparative analysis of Acinetobacter baumannii clinical isolates: insights from genomic and phenotypic approaches

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Introduction: Clinical isolates of Acinetobacter baumannii have undergone significant evolutionary pressure due to extensive exposure to stress conditions, particularly with the widespread use of antibiotics. This pressure has led to local clonal expansion and adaptation to various environmental and host factors. Therefore, this study aimed to compare the resistance, virulence, and phenotypic properties of clinical isolates collected over the last decade, employing genomic and phenotypic approaches. Materials and methods: Thirty clinical isolates were obtained from respiratory and urine specimens collected between 2010 and 2023 at a public hospital in Rome. Whole genome sequencing was performed using the Illumina MiSeq platform. Data analysis was conducted using ResFinder, ISfinder, and PlasmidSeeker. Sequence Types (STs) were assigned using the PubMLST Pasteur scheme. Antibiogram and main phenotypic characteristics were also assessed. Results: All isolates belonged to ST2, except one belonging to ST632. Variants of aminoglycoside resistance genes were more prevalent in past respiratory isolates. The blaOXA-23 gene was absent only in two past respiratory isolates. blaOXA-51 and blaADC variants were found in all isolates. blaTEM-1 was detected only in current respiratory isolates, whereas ftsI_A515V was dominant in recent respiratory isolates and in all urine isolates. Genes encoding efflux pumps were found in all isolates. Notably, heavy metal efflux pumps arsC and merE were found only in one past respiratory isolate. ISAba125 was predominant in old respiratory isolates. Strains lacking tsap were not motile. Discussion and conclusion: The recent respiratory isolates exhibited a higher content of antibiotic-resistant and virulence genes. Despite displaying lower MIC values, they were more invasive compared to earlier isolates. These strains showed the highest macrocolony morphology variability. Differently, urinary isolates remained consistent across the different collection times. Investigation of plasmid content is ongoing.

189 - FUNCTIONALITY OF HUMAN LIVER MACROPHAGES IN THE UPTAKE AND CLEARANCE OF BACTERIA DURING EX VIVO ORGAN PERFUSION

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Functionality of human liver macrophages in the uptake and clearance of bacteria during ex vivo organ perfusion

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Tissue-resident macrophages are a heterogeneous population that provide innate cellular immunity by carrying out clearance of pathogens from the bloodstream and protect against sepsis during systemic infections. Previous studies in mouse models have shown that invasive bacterial disease occurs after rare events of failure of macrophage function that led to replication of bacteria within macrophages and subsequent systemic infection. Notably, recent data described that murine hepatic Kupffer cell macrophages were successful in clearing Streptococcus pneumoniae in a serotype-dependent manner.

To test whether this observation in mice corresponded to similar mechanisms in humans, human liver segments (ClinicalTrial.gov identifier: NCT05255042 and NCT04620824; REC 21/PR/0287 and REC 18/EM/0057 respectively) were infected with a cocktail of S. pneumoniae with strains with high and low potential for invasive disease in an ex vivo perfusion system. Human livers were harvested, infected with 107 cells, and perfused for 6 hours. Biopsies and perfusates were collected at different time points and processed for viable bacterial cell counts. Biopsies were also stained for immunofluorescence for macrophages, cell apoptosis, and bacterial markers.

Viable bacterial cell counts from both biopsies and perfusates showed an increase in bacterial load over time. No significant differences were observed in the clearance of low- and high-virulence

serotypes. Microscopy image analysis revealed no changes in the apoptosis marker signal in Kupffer cells during the infection period, as less than 1% of the apoptosis marker colocalized with macrophages in both infected and uninfected livers at all time points. Furthermore, high- and low-virulence serotypes colocalized with hepatic macrophages without statistical differences, consistent with the viable bacterial count results.

Human hepatic macrophages show low efficiency in pneumococcal clearance, and no significant differences were observed between the uptake of low- and high-virulence serotypes with the bacterial dose tested. These findings suggest that the role of liver tissue resident Kupffer cell macrophages in invasive pneumococcal infection differs between murine and human livers.

202 - OUTCOMES OF UROPATHOGENIC ESCHERICHIA COLI AND INFLUENZA VIRUS COINFECTION IN BLADDER CELLS

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Outcomes of uropathogenic Escherichia coli and Influenza virus coinfection in bladder cells

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Introduction. Urinary tract infections (UTIs) are the most common diseases encountered in clinical practice worldwide and account for morbidity and high medical costs. Uropathogenic Escherichia coli (UPEC) are responsible for the vast majority of UTIs. Multiple studies have reported an association between respiratory viruses and UTIs and concomitant infections by two or more pathogens are often observed. Notably, as reported by some authors, influenza A viruses (IAV) have been isolated from kidney tissue in human autopsies. Furthermore, IAV could be detected in the urine of patients during several pandemic outbreaks. For these reasons, in order to obtain further information on mixed viral-bacterial infections in the genitourinary tract, in this study the interaction between IAV-infected bladder cells and UPEC strains was investigated.

Materials and Methods. UPEC and reference strains were evaluated for the ability to adhere, invade and survive in T24 bladder cells monolayers by gentamicin protection assay. Cell monolayers were infected with different influenza viruses: human A/Puerto Rico/8/34 H1N1 (PR8/H1N1), human A/California/7/2009/H1N1 (pH1N1) or avian Parrot/Ulster/73 H7N1 (H7N1) strains. Viral titre was analyzed by Hemagglutination assay (HAU) 24 h post infection (p.i.). For the co-infection experiments, bacteria were added in the last hour of PR8 replicative cycle. At 24h p.i. viral release was assessed by RT-qPCR in culture supernatants and viral proteins were analyzed by western blot assay in cell lysates.

Results. UPEC strain was able to efficiently adhere to and invade human bladder cells. Similarly, these cells were permissive to all influenza virus strains studied, as demonstrated by viral production released in the supernatants of infected cells. Co-infection experiments showed that, when the monolayers were pre-infected with the virus, a decrease in the internalization of UPEC strain in T24 cells was detectable. Interestingly, PR8/H1N1 viral RNA in cell supernatants was reduced when the cells were infected with bacterial strains. Furthermore, viral protein expression (hemagglutinin, HA nucleoprotein, NP, matrix protein 1, M1) was affected by bacterial infection.

Discussion and Conclusions. These preliminary results suggest a possible interaction between viruses and bacterial cells, probably during the early phase of bacterial adhesion to the bladder cells. Indeed, as already reported by some authors, sialic acids are involved in bond ability of E.coli strains. Further studies are necessary to better understand the complex functional interactions between viruses/bacteria and host cells, that will allow the development of new specific diagnostic and therapeutic approaches.

204 - CLN8 PROTEIN MAY BE IMPLICATED IN THE ENDOCELLULAR TRANSLOCATION OF CYTOLETHAL DISTENDING TOXIN PRODUCED BY CAMPYLOBACTER JEJUNI.

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CLN8 protein may be implicated in the endocellular translocation of cytolethal distending toxin produced by Campylobacter jejuni.

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INTRODUCTION: Campylobacteriosis (CPb) is one of the most common gastrointestinal bacterial diseases in the world. Moreover, the CPb is one of the risk factors for the development of secondary sequela such as the Guillain–Barré syndrome. The acute consequences of CPb have been attributed to the toxic activities of the cytolethal distending toxin (CDT) on mucosal cells. The CDT interacts with host cell surface at membrane lipid rafts binding the cholesterol through subunit A and C, two of subunits of trimeric CTD. The third active subunit, CTD-B, is internalized after the interaction with Cellugyrin and translocated inside endo-cellular vesicles from the cell membrane; according to recent observations, this step is a requisite in the earliest stages of CDT internalization. Recently, it has been demonstrated that low expression of CLN8 gene is associated with a low risk of symptomatic CPb. CNL8 is described as a protein involved in the ceroid neuronal lipofuscinosis (CNL), one of lysosomal storage diseases. Homozygous mutations in CLN8 gene, located in the chromosome 8, lead to the development of CNL. CLN8 is a protein involved in endocellular translocation of the lysosomal enzymes, although all its functions are not yet completely known. In a recent work the genetics of asymptomatic campylobacter carriers was studied. The authors demonstrated a lower risk of developing symptomatic gastroenteritis in subjects carrying different allele (SNP G>A) in the region on chromosome 8 in the ARHGEF10 gene. This SNP is associated with decreased expression of the neighboring gene, CLN8, suggesting that CLN8 may be involved in the intracellular translocation of CDT. In our work, we developed a CLN8null (C8n) human cell line to test the key role of CLN8 in CDT cell response.

Material and Methods: the C8n cell line was obtained using CRISPR; C. jejuni ATCC 29428 was employed to prepare lysate (jL); in comparison with the parental line (PL) C8n cell was treated with equivalent amount of jL to analyze: i) distending effect, ii) WB to Bcl2 and iii) ATP production

Results: The models cell line responds to jL treatment to: i) distending effect, minimal in C8n line; ii) content of Bcl2, decrease in PL and the opposite trend in C8n line, and iii) amount of ATP, decrease in PL while stationary in C8n cell

Discussion AND conclusion: These preliminary results match the comparative genomic data indicated above, suggesting the key role of CLN8 in the clinical development of infectious disease. The discovery of CDT endocellular pathway to achieve the final intracellular target developing toxicity could indicate new approaches in acute infectious disease. Further experiments are needed to identify the mechanisms by which CLN8 interacts with the CDT subunit B.

208 - DYNAMICS OF PARVOVIRUS B19 AND CELLULAR TRANSCRIPTOME IN INFECTED, DIFFERENTIATING ERYTRHOID PROGENITOR CELLS

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Dynamics of Parvovirus B19 and cellular transcriptome in infected, differentiating erytrhoid progenitor cells

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Introduction

Parvovirus B19 (B19V) is a human pathogenic virus with a marked tropism for erythroid progenitor cells (EPCs). A single pre-mRNA is processed into five mRNA classes, whose alternative forms depend on the diverse combinations of alternative splicing and cleavage/polyadenylation sites. Further, the impact of B19V on EPCs, eventually leading to cell death, is not completely understood. In our work, we used mRNAseq to investigate the virus expression profile and the induced effects on host cell expression profile in B19V infected, differentiating EPCs

Materials and Methods

In vitro-expanded and differentiated EPCs were infected with B19V, and cell fractions were collected at different hours post infection (hpi). The amount of viral DNA and of different subsets of viral mRNAs were assessed by qPCR and qRT-PCR; total DNA was analysed by genomic HTS; poly-A mRNA was selected and analysed by mRNAseq. Obtained reads were aligned on reference viral and human genomes for mapping and differential expression analysis.

Results

qPCR and qRT-PCR confirmed the productive infection of EPCs and a biphasic early/late pattern of viral transcription. By genomic DNA HTS, the viral genome was identified accounting for <1% of total

reads at 48 hpi. Viral mRNAs were identified, accounting for 20% of total reads at 48 hpi; selective mapping of reads allowed to reconstruct the relative frequency of usage of pre-mRNA processing signals and the resulting viral transcriptome.

Concerning the cellular transcriptome, we identified around 90 dysregulated genes in samples taken at 2hpi and 16hpi. In the 48hpi samples, there were approximately 80 dysregulated genes. Among these, 38 genes were consistently dysregulated across all three time points.

Discussion and Conclusions

Our analysis allowed recostruction of the viral transcriptome and investigation of virus-induced cell expression dysregulation in EPCs. The pathways implicated in B19V infection have been shown to intersect with cellular senescence and the cell cycle, underscoring the impact of virus on cell physiology andits implications for pathogenetic mechanisms. Translating analysis to in vivo conditions will provide insights into the virus's mechanisms within the body and its potential implications for diseases and medical conditions.

214 - EXPLORING ESSENTIAL GENES IN STAPHYLOCOCCUS AUREUS JE2-MRSA BY TRANSPOSITION- DIRECTED INSERTION SITE SEQUENCING (TRADIS)

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Exploring Essential Genes in Staphylococcus aureus JE2-MRSA by: Transposition- Directed Insertion Site Sequencing (TraDIS)

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Introduction: Methicillin resistant Staphylococcus aureus (MRSA) is a major public health concerns worldwide causing a variety of clinical diseases including sepsis and abscess/skin infections. Transposon Directed Insertion-site Sequencing (TraDIS) is a genome-wide mutagenesis method, which allows simultaneous assaying of large transposon mutant libraries for exploring gene significance, functionality, and inter genetic relationships in bacteria. In the current study, our goals were i) to construct a high-density Tn-mutants library in the JE2-MRSA strain of this species, and ii) to identify the essential genes for in growth in ex vivo human blood and for in vivo infection of skin and organs in a mouse model.

Materials and Methods: We transducted the mariner HimarI transposon/transposase complex into JE2-MRSA strain by phage ø11 using an improved transduction protocol to create a high-density transposon library of 10^9 mutants. Following the library was submitted for survival and skin/systemic infection investigations in human blood and murine model respectively. The bioinformatic analysis for output and input libraries was performed by Bio-Tradis analysis pipeline.

Results: We identified 289, 123, and 106 essential genes for survival in human blood, systemic and skin infection respectively. Purine and pyrimidine encoding genes are essential for both skin and systemic infection while genes responsible for meanquinone and chorismate synthesis are dominant in blood survival and systemic infection as well.
Conclusion: Our study provides a comprehensive analysis for the metabolic genes involved in the survival of JE2-MRSA in human blood and both systemic and skin infection models which could be a promising potential drug targets against MRSA infection.

222 - DECIPHERING THE ROLE OF CODY IN CONTROLLING EXPRESSION OF SRR2, A CRUCIAL ADHESIN FOR GROUP B STREPTOCOCCUS VIRULENCE

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Deciphering the role of CodY in controlling expression of Srr2, a crucial adhesin for Group B Streptococcus virulence

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Introduction

Group B Streptococcus (GBS) poses a significant threat as an opportunistic pathogen, particularly affecting newborns, pregnant women, and vulnerable individuals. Its ability to cause severe infections like pneumonia, sepsis, and meningitis is attributed to various virulence factors, with the cell wall-anchored serine-rich repeat glycoprotein Srr2 playing a crucial role in mediating virulence and host interaction. By binding to plasma proteins, Srr2 facilitates bacterial dissemination. Furthermore, it aids in adhesion to brain endothelial cells, promoting invasion of the blood-brain barrier, particularly in newborns. Understanding the regulatory and signaling systems controlling the expression of GBS virulence factors is essential to elucidate the pathogenesis of GBS infection and identify novel therapeutic agents.

Materials and Methods

Here, we investigated the role of the global transcriptional regulator CodY in controlling the expression of srr2 in GBS. Using DNA-binding assays, expression studies, and mutational analyses, we elucidated the regulatory mechanisms underlying CodY-mediated repression of srr2 during exponential growth in rich medium.

Results

Our findings revealed that CodY directly binds within the regulatory region of the srr2 gene, exerting negative control over its expression. Furthermore, we observed a significant interplay between CodY and CovR, another important transcriptional regulator, in regulating srr2 expression, shedding light on the complex regulatory network governing GBS pathogenesis.

Discussion and Conclusions

The elucidation of CodY-mediated regulation of srr2 provides valuable insights into the molecular mechanisms underlying GBS virulence and host interaction. Understanding how CodY coordinates with other regulatory factors in controlling key virulence determinants like Srr2 is essential for unraveling the complexities of GBS pathogenesis. These findings pave the way for developing targeted therapeutic interventions aimed at disrupting GBS-host interactions and mitigating the impact of GBS-associated infections.

225 - PARVOVIRUS B19 GENOMIC SEQUENCE DIVERSITY AND SHIFTING EVOLUTION

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Parvovirus B19 Genomic Sequence Diversity and Shifting Evolution

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Introduction

Parvovirus B19 (B19V) is a widely distributed human ssDNA virus responsible for an ample range of clinical manifestations. Although credited with a high genetic substirution rate, the amount of information on sequence diversity is low compared to its wide diffusion and a comprehensive picture of its genetic diversity is lacking. The aim of the present work was to investigate B19V genomic patterns of variability and diversification by High-Throughput Sequencing (HTS), making use of a dedicated bioinformatic tool and analysis pipeline.

Materials and Methods

A set of 24 high-titre viraemic samples collected in the metropolitan area of Bologna in the period 2014-2024 were analysed. Samples were processed by whole-genome target enrichment, then sequenced on Illumina NovaSeq 6000 in paired-end mode, with reads of 150 bps. Quality-processed reads were aligned on the reference consensus B19V EC genotype 1 sequence (KY940273.1), then analysed by an established algorithm and in-house developed bioinformatic tool, QuasiSpecies Analyser (QSA), to assess intra-sample sequence diversity. K-means clustering and PCAwere used to assess inter-sample sequence diversity and temporal clustering.

Results

For intra-sample sequence diversity, QSA yielded both position-specific and cumulative Shannon Entropy values, combined in a diversity index tagged as α-diversity, related to the frequency of geentic diversity. Our results quantify a low sequence heterogeneity, with α-diversity values in a range 1.2-8.0 10-3, lower than previoulsy reported but in accordance with replication operated by cellular DNA polymerases. A diversity index, tagged as δ-diversity, was elaborated as a measure of genetic distance between any isolate pair, incorporating both intra-sample and inter-sample sequence heterogeneity.

Use of δ-diversity, coupled to K-means analysis, yielded distinct isolate clusters related to timing of isolation, with PCAconfirmed the complementary contribution of α- and δ-diversity in defining the different clusters.

Discussion and Conclusions

Our analytical approach elaborates on information derived from HTS into assessment of both intra-sample and inter-sample genetic diversity, and shows advantage over consensus-sequence based analytical tools. Specifically relating to B19V, our results indicate a low genetic diversity and substitution rate, and a prevailing shifting mode of evolution, by progressive replacement of closely related isolates in subsequent epidemic cycles.

231 - ROLE OF MATRIX METALLOPROTEINASES IN INFLUENZA A VIRUS INFECTION OF NEURONAL CELLS

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Role of matrix metalloproteinases in influenza A virus infection of neuronal cells.

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Introduction: Several intracellular factors affect the outcome of viral infection; on the other hand, viruses induce considerable alterations in cellular homeostasis. While multiple pathways and their modifications have been deeply described during influenza A virus (IAV) infection in their target cells, respiratory epithelial cells, less is known about these modifications in infected neuronal cells which, although not the main target, can be reached by IAV through different routes leading to nervous complications. Cell modifications in IAV-infected epithelial cells include the up-regulation of several redox-dependent enzymes, including matrix metalloproteinases (MMPs), endopeptidases involved in the inflammatory process. The aim of this study was to analyse the role of MMPs in influenza virus replication and viral-induced inflammation in a neuronal model of infection.

Materials and Methods: Human neuroblastoma SH-SY5Y cells were differentiated from the initial epithelial-like into neuronal phenotype; then they were infected with influenza A/NWS/33 H1N1 (NWS) at high or low m.o.i. of infection for 8-24 h. The supernatants were collected and analyzed for the viral titer and infectivity by hemagglutinin assay, reverse transcription-quantitative PCR (RT-qPCR) and TCID50; for the MMPs activity gelatin zymography was used. Cell lysates were analyzed for viral proteins, MMPs and cytokines expression, both at mRNA and protein levels, by RT-qPCR and immunoblotting with specific antibodies, respectively.

Results: NWS virus, that efficiently infects and replicates in differentiated SH-SY5Y cells, induced an increase in MMPs, particularly of MMP-2 and MMP-9, and in cytokines (IL-1 β, TNFα, IFN β) expression. In the supernatants of NWS-infected cells, we observed an increase in both MMPs enzymatic activity. The treatment with MMPs inhibitor, Batimastat, led to a reduction in viral replication and to a decrease in cytokines expression. Interestingly, a redox-modulating agent, 2-AAPA, that we already showed to reduce inflammatory cytokine IL-6 secretion from epithelial cells, reduced secreted MMPs activity,

suggesting that redox dependent secretion of these enzymes could contribute to the virus replication and spread.

Discussion and Conclusions: The results showed the involvement of MMP-2 and MMP-9 in infection of neuronal cells by influenza A virus and in the inflammatory response to the virus, moreover suggesting that redox-sensitive pathways could be involved in enzyme secretion and activity. Further study is in progress to investigate the secretory pathways and the mechanisms underlying the effect of MMPs on viral replication and virus induced inflammation in the nervous system.

239 - CHARACTERIZATION OF PATHOGENIC MECHANISMS DURING INFLUENZA VIRUS AND HCOV-229E INFECTIONS BY USING AIR-LIQUID INTERFACE MODELS

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Characterization of pathogenic mechanisms during influenza virus and HCoV-229E infections by using air-liquid interface models

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Introduction: Understanding the pathogenic mechanisms of respiratory virus infections is a crucial point to address the emergence of new viral strains. Changes in redox state have been identified as a key event in regulating viral replication. Indeed, the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), involved in the antioxidant response, negatively impacts influenza virus replication. However, there is a limited understanding of how redox changes can influence viral replication in different respiratory tract compartments. The aim of this study was to characterize the intracellular redox alterations during influenza and coronavirus infections in alveolar and bronchial cells by using air-liquid interface (ALI) models.

Materials and Methods: Bronchial cell line (BEAS-2B), primary bronchial cells (NHBE) or alveolar cell line (A549) were infected with influenza type A/Puerto Rico/8/34 H1N1 (PR/8), A/California/7/2009/H1N1 (pH1N1), A/parrot/ulster/73 (H7N1) strains or seasonal coronavirus (HCoV-229E). NHBE cells were grown in ALI and after 21 days of differentiation, validated by immunofluorescence analysis, were infected. At different timepoints post infection (p.i.), the Nrf2-antioxdant response was analyzed by measuring mRNA levels of different Nrf2 target genes by RT-qPCR and the corresponding proteins by western blot. Glutathione (GSH) was measured by HPLC. Finally, cells were infected and treated with the pro-GSH compound, I-152. The nuclear/cytoplasmic extracts were analyzed for the expression of Nrf2 by western blot, while the viral titer was evaluated by TCID50.

Results: In bronchial cells infected with HCoV-229E, we observed an increase of viral replication starting from 6 h p.i., which correlated with a slight decrease of Nrf2 and Heme-Oxygenase 1 at transcriptional level. NHBE cells were fully differentiated in a pseudostratified epithelium and a marked cytopathic effect was visible after 24 h p.i., along with a redox alteration. Moreover, our data showed that distinct basal GSH levels in bronchial and alveolar cells were associated with different permissiveness to viral infections. Indeed, we found higher replication in bronchial cells where basal GSH levels were lower. Finally, we observed that Nrf2 activation correlated with a decrease of viral replication in both influenza virus and HCoV-229E infected cells treated with I-152.

Discussion and Conclusions: Our data showed a key role of the Nrf2-antioxidant response in regulating different viral infections in distinct respiratory tract compartments. Further studies will allow to define the role of Nrf2 pathway in contributing to severe conditions during respiratory virus infections by using innovative models that mimic the epithelial-endothelial barrier.

244 - HSV-1 INFECTION INDUCES DYRK1A ACTIVATION AND CONSEQUENT TAU PHOSPHORYLATION IN HUMAN NEUROBLASTOMA CELLS

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Topic: Patogenesi e meccanismi di virulenza microbici

HSV-1 infection induces Dyrk-1A activation and consequent tau phosphorylation in human neuroblastoma cells

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Background and aims: Among the environmental risk factors for Alzheimer's disease (AD) onset and progression, a growing body of evidence supports the involvement of herpes simplex virus type-1 (HSV-1) infection/reactivations reaching the brain. We previously reported that HSV-1 multiple reactivations induce the accumulation of amyloid-β peptides and the hyperphosphorylated forms of tau protein (ptau) in the brain of infected mice. The accumulation of such proteins, hallmarks of AD pathology, results from an altered and complex balance among several kinases/phosphatases/secretases that regulate their post-translational modifications. In particular, tau phosphorylation is driven by many kinases such as GSK3-beta, CDK5, and DYRK1A, which may act synergically on the same phosphorylation sites of the protein or specifically target selected ones. However, which are the kinases underlying the virus-induced ptau is still controversial. We previously showed that HSV-1 infection in vitro induces the activation of GSK3-beta leading to APP phosphorylation. Here we focused on HSV-1-induced tau phosphorylation investigating the possible involvement of DYRK1A.

Methods: Levels of ptau in HSV-1- and mock-infected SH-SY5Ycells were evaluated at different times post-infection (p.i.) by Western blotting with antibodies directed against specific phosphorylated sites of the protein and total tau. The efficacy of HSV-1 infection was evaluated by standard plaque and in-Cell western assays. Inhibitors of DYRK1A (designed and synthesized by Perha Pharmaceuticals), and

of GSK3-beta were used to treat HSV-1- and mock-infected cells within the absorption phase and for 24h p.i. MTT assay was used to check their cytotoxicity on SH-SY5Y cells.

Results: We found that in human neuroblastoma cells: 1) HSV-1 induced tau phosphorylation at threonine 217 (T217), directly targeted by DYRK1A and GSK3-beta, and serine 214 (S214), that is not a DYRK1A target; 2) HSV-1 infection induces the cleavage of DYRK1A protein in its active form shortly before tau phosphorylation at T217; 3) the inhibitor of DYRK1A was effective in inhibiting HSV-1-induced cleavage on the kinase and phosphorylation of tau at T217 in a dose-dependent manner, whereas its inactive form, used as control, was ineffective; 4) GSK3-beta was not directly involved in HSV-1-induced tau phosphorylation at T217 since its inhibitor did not affect such event.

Conclusions: Overall, these results indicate that HSV-1 exploits DYRKA to induce tau phosphorylation at T217 in human neuroblastoma cells and suggest DYRK1A as possible target for counteracting HSV-1-induced neurodegeneration.

245 - H1N1 INFLUENZA VIRUS INDUCES MARKERS OF NEURODEGENERATIONS IN NEURONS WITHIN IN VITRO ACUTE INFECTION

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Topic: Patogenesi e meccanismi di virulenza microbici

H1N1 influenza virus induces markers of neurodegenerations in neurons within in vitro acute infection

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Introduction: H1N1 influenza A virus (IAV), responsible for both seasonal epidemics and worldwide severe pandemics, usually infects the epithelial cells of the upper respiratory tract but can reach the lower respiratory one, leading to more severe clinical manifestations such as respiratory distress syndrome and pneumonia, especially in vulnerable individuals. In some subjects, IAV infection can also affect the central nervous system, causing acute neurological manifestations, mainly mediated by neuroinflammation, and possible long-term sequelae that have been related to neurodegenerative diseases, including Alzheimer's disease (AD). Recent findings from in vivo studies in an AD transgenic mouse model suggest that IAV infection may accelerate the disease progression. On the contrary, some papers report that amyloid-beta peptides (Abeta), 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.

Methods: Primary cultures of murine neuron-glia co-cultures and human neuroblastoma SH-SY5Y cells were infected with different doses of IAV or mock solution and analyzed at various times post infection (p.i.). Cells were analyzed by Western Blot (WB), confocal immunofluorescence (IF) and RT-PCR for assaying the intracellular expression levels of different proteins related to neurodegeneration and neuroinflammation, such as Amyloid Precursor Protein (APP), Abeta, tau and its phosphorylation (ptau), the post-synaptic markers PSD-95, interleukin-1beta (IL-1beta) and caspase-1.

Results: Our results show that IAV infection induced APP downregulation and an accumulation of Abeta in both primary brain cells and SH-SY5Y cells, suggesting that the virus infection may promote APP amyloidogenic processing. We also found an increase in intracellular IL-1beta following IAV infection that was paralleled by the activation of caspase-1. On the contrary, IAV did not induce a significant increase 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. We also found that

the virus infection induced a significant decrease of PSD95, indicating that the virus induces synaptic damage within the acute infection.

Conclusions: Overall, our results indicate that IAV infection affects neuron physiology within the acute infection, possibly leading to neurodegeneration. Further studies are required to unravel the role of cell response in such events as well as their long-term potential.

250 - THE BURDEN OF CANDIDA PARAPSILOSIS BLOODSTREAM INFECTIONS: FROM AZOLE RESISTANCE TO BIOFILM PRODUCTION.

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The burden of Candida parapsilosis bloodstream infections: From azole resistance to biofilm production.

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INTRODUCTION: The aim of our work was to evaluate the incidence and antibiotype of Candida parapsilosis strains isolated from blood cultures at our hospital, the possible correlation between phenotypic and genotypic fluconazole resistance, and their ability to produce biofilm. MATERIALS AND METHODS: C. parapsilosis strains were isolated from blood cultures by the BACTEC broth culture system and phenotypically identified by mass spectrometry (MALDI-TOF). Antifungal susceptibility testing (AST) was determined by broth micro-method (Sensititre YeastOne Colorimetric Broth). AST reading was performed using Clinical & Laboratory Standards Institute (CLSI) guidelines for the interpretation of Minimum Inhibitory Concentration (MIC) values. The different strains isolated were distinguished into three groups: Group 1 which comprised the strains susceptible to fluconazole (MIC <= 2 mcg/ml) and voriconazole (MIC <= 0.12 mcg/ml), Group 2A including the strains resistant to fluconazole (MIC >= 8 mcg/ml) and voriconazole (MIC >= 1 mcg/ml) and Group 2B into which isolates were resistant to fluconazole (MIC >= 8 mcg/ml) and intermediate to voriconazole (MIC 0.25-0.5 mcg/ml). The study of genotypic resistance was conducted by Sanger sequencing of the ERG11 gene. Biofilm production was evaluated according to Ramage's method, and a biofilm score (BS) was assigned as follows: low (1+,2+), medium (3+,4+) and good (5+,6+) producers. RESULTS: ERG11 gene sequencing of 46 strains isolated in 2021 (46/100) mostly showed the presence of the Y132F and I197I (synonymous mutation) while the R398I and I261M mutations were quite rare with no apparent phenotypic resistance. Of these 46, 74% belonged to groups 2A and 2B, furthermore these strains showed low BS biofilm production (1+,2+). Regarding the biofilm analysis, a total of 71 C. parapsilosis strains were evaluated, of which 73.24 % (52/71) belonged to groups 2A and 2B and were associated with low BS (1+/2+) while 26.7 % (19/71) belonged to group 1 and expressed various levels of biofilm

production (10/19 were medium and good BS producers). DISCUSSION AND CONCLUSIONS: The results obtained show that the strains harboring the Y132F mutation correlate with the phenotypic resistance to fluconazole whilst the I197I synonymous mutation does not impact the morphology of the target region and thus no associated resistance is observed. Additionally, it can be hypothesized that azoles, not being endowed with anti-biofilm activity, unlike echinocandins and liposomal amphotericin B, may have reduced efficacy in vivo, even against the phenotypically susceptible strains with medium and good BS scores. This can be especially true in cases of catheter-related candidemia.

257 - THE TAXONOMIC STRUCTURE OF LUNG-ASSOCIATED MICROBIOTA CAN MODULATE TUMOR MICROENVIRONMENT.

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The taxonomic structure of lung-associated microbiota can modulate tumor microenvironment.

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Background - Emerging evidence revealed the existence of a specific lung microbiota that plays a key role in immunotolerance through the induction of Tregs. Recent findings suggested lung tumors to be characterized by a different microbiota compared to the normal tissue but current research on the intra-tumoral microbiota involvement in the genesis, progression, and drug response is still embryonic. In the present study, we evaluated the composition of the local microbiota in C57BL/6 mouse lungs bearing Luis Lung Cancer 1 (LLC1 carcinoma), and the effect of antibiotic administration in tumor microenvironment modulation.

Methods - Lung-colonizing bacteria were characterized by culturing homogenates from healthy and tumoral lungs under aerobic, anaerobic and microaerophilic conditions. Isolated strains were identified by MALDI-TOF (BioMerieux VITEK MS). To better clarify the composition of the microbiota, the same tissues were subjected to 16S rRNA gene sequencing. Based on the ability of some gut bacteria to induce immunosuppressive cells, we evaluated, by flow cytometry, the capability of supernatants obtained from isolated bacteria to induce Tregs.

Results - We grew different bacteria strains from tumor-bearing lungs: S. capitis, E. faecalis, K. oxytoca, K. aerogenes, E. coli, S. epidermidis and S. aureus. Notably, we did not isolate any bacteria from healthy lungs, likely related to their very low biomass. NGS analysis showed an enrichment in tumor tissues of amplicon sequence variants belonging to Bacterioidales, Clostridiales, Lactobacillales and Enterobacteriales in LLC1 tumor-bearing lungs. Local treatments with aerosolized vancomycin/neomycin starting 4 days after tumor injection reduced the relative abundance of those taxa. Some of the viable bacteria isolated from tumoral tissues were able to induce the expansion of Tregs in vitro. Here again, antibiotics strongly dropped Treg frequency.

Conclusions - Our results suggest that tumor growth shapes lung microbial composition by promoting the expansion of specific microorganisms. The evidence that antibiotics might reverse tumor-specific taxa overgrowth can pave the way to future experiments aimed at deciphering the therapeutic potential of localized antibiotic treatments to reverse the immunosuppressive microenvironment.

258 - LET'S COMMUNICATE: EXTRACELLULAR VESICLES IN C. ALBICANS

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Let's communicate: extracellular vesicles in C. albicans

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Background-Extracellular vesicles (EVs) are an increasingly important research field to understand how cells can modulate the response to environmental stimuli. Microbial EVs can deliver virulence factors and effector molecules to host cells modulating immune responses and promoting fungal colonization and infection. Candida albicans produces and releases EVs that can be involved in biofilm formation and drug resistance. As with the cancer microenvironment, elucidating the role of EVs in fungal pathogenesis maypave the way for the development novel antifungal strategies.

Material and Methods- The CAF2.1-dTomato strain of C. albicans, carrying the pENO1-dTom-NATr plasmid, was used in this study. The plasmid contains a codon-optimized version of the dTomato gene under the control of the constitutive ENO1 promoter. CAF2.1-dTomato was cultured under dynamic (planktonic) and static (biofilm) conditions at 37°C in 40 ml of RPMI medium. After 24 hours, cells were harvested by centrifugation at 800 g at room temperature. The supernatants were ultracentrifuged twice at 20000g for 30 min, and then at 100000 x g for 90 min. The size and the concentration of the EVs were assessed using the nanoparticle tracking analysis (NTA- Nanosight NS300, Malvern Panalytical). Fluorescence analysis was performed using a fluorescence microscope (Zeiss, Germany).

Results- The concentration of EVs appeared higher in the planktonic condition compared to sessile cells (6x108 particles/ml vs 3x108 particles/ml, respectively). We found no differences in the size of EVs, which ranged from 80nm to 90nm in diameter. Notably, we observed a fluorescence signal in EV suspensions, probably derived from the constitutive ENO1 promoter, which modulates the expression of the dTomato gene in EVs.

Discussion and Conclusions- According to literature, we observed a significant production of EVs in our experimental protocol. Growth conditions do not seem to affect the concentration and the size of the EVs in C. albicans. The observed fluorescence of the vesicles suggests a possible inherited component of the cell wall. Future experiments will focus on the functional characterization of planktonic and sessile EVs and to understand whether biofilm-derived vesicles can induce biofilm formation in clinical isolates of Candida with low propensity to adhere.

284 - PATHOGENETIC IMPLICATIONS OF STAPHYLOCOCCUS AUREUS EXTRACELLULAR VESICLES IN THE CONTEXT OF SKIN DYSBIOSIS

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Pathogenetic implications of Staphylococcus aureus extracellular vesicles in the context of skin dysbiosis

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Introduction. Atopic dermatitis (AD) is a chronic skin condition characterized by skin dryness, itching, and eczematous lesions. This disease is related to a dysregulation of the immune system and microbial colonization, particularly of Staphylococcus aureus, at the level of the inflamed skin, contributing to the worsening clinical condition of the AD patient. Proteomic analysis identified several immune evasion proteins and virulence factors in the extracellular vesicles (EVs) of S. aureus, suggesting a possible role of these proteins in AD’s pathogenesis. The objective of this study was to evaluate the potential efficacy of a wall fragment obtained from a patented strain of C. acnes DSM28251 (c40) and its combination with a mucopolysaccharide transporter (HAc40) in counteracting the cytotoxicity of EVs produced by S. aureus ATCC 14458.

Materials and Methods. The polylysine method isolated EVs of S. aureus ATCC 14458 and characterized by proteomic analysis by liquid chromatography-tandem mass spectrometry. In vitro studies on cell lines were conducted to evaluate c40 and HAc40 treatment.

Results. Proteomic analysis revealed significant protein content in EVs, mainly in the cytoplasm. In addition, in vitro studies on cell lines showed that HAc40 and c40 treatment modified the pathogenicity of S. aureus EVs. This was confirmed by gene expression analysis of the splicing proteins

zona occludens 1 (ZO1) and claudin 1 (CLDN1), indicating their protective function on the epidermal barrier.

Discussion and Conclusions. The in vitro treatment with Hac40 and c40 can reduce the harmful effects of S. aureus EVs. Future studies may elucidate the mechanisms underlying this interaction and explore the potential clinical utility of c40 and HAc40 in managing AD.

286 - REARRANGEMENTS IN A PE_PGRS GENE LOCUS AND IMPACT ON THE EVOLUTION OF MTB

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Rearrangements in a pe_pgrs gene locus and impact on the evolution of MTB

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Introduction. Sequencing of Mycobacterium tuberculosis (Mtb) genome allowed the characterization of two gene families named pe/ppe encoding glycine-rich proteins and properly named as PE and PPE proteins. Both pe and ppe genes are found in pathogenic and saprophytic mycobacteria, while pe_pgrs genes are significantly more represented in Mtb complex. However, the functional consequences of pe_pgrs gene expansion and diversification in Mtb remain poorly understood. pe_pgrs genes are commonly found as single gene dispersed in the genome, but few, including pe_pgrs3 and pe_pgrs4 are located nearby on the genome. In this study, we aimed to investigate, using publicly available genome sequences from representative MTB complex strains, the evolution of the pe_pgrs3/4 gene locus and infer the potential consequences on Mtb transmission and success.

Materials and Methods. We analyzed ~300 Mtb complete genomes to study genomic organization of pe_pgrs3 (Rv0278c, 2874 bp) and pe_pgrs4 (Rv0279c, 2514 bp) genes and their evolution in Mtb complex. We searched for paralogues genes of the pe_pgrs3 and pe_pgrs4, focusing the analysis on the characterization of the genetic events that led to the emergence of the unique sequence coding the C-terminal polybasic domain and on the duplication and rearrangements of these two highly homologous genes.

Results. The genomic locus containing the pe_pgrs3/pe_pgrs4 genes was retrieved by Mtb complete genomes and associated to each strain previously characterized in terms of lineage identification. NJ-tree was constructed by using cgMLST to highlight how locus evolution follows Mtb lineages. In one of

the outgroups (M. canettii) analyzed we could not find any of the two genes, while in a second M. canetti we detected a single gene, ancestral of the pe_pgrs3/4. In Mtb we observed a duplication leading to the appearance of the locus containing three genes (pe_pgrs3a/3b/4) that is conserved in most lineages apart from the two modern lineages 2 and 4, where most of the Mtb strains possess two genes (pe_pgrs3/4). Interestingly, the duplication events and rearrangements observed among the Mtb strains analyzed did not produce significant genetic variability, except for an insertion of 1 bp that prompted a frameshift in the PGRS coding sequence that leads to the expression of an arginine-rich protein sequence instead of a glycine-rich region.

Discussion and conclusions.

This study highlights the mechanisms of genetic rearrangements in the pe_pgrs genes in Mtb and how certain genotypes were associated with the most successful clades of the Mtb modern lineages. Our data support the importance and potential consequences in the emergence of the sequence coding the arginine-rich region in PE_PGRS3, possibly warranting access to phospholipids that are necessary for the replication of Mtb in host macrophages.

T05 PATOGENI EMERGENTI E RIEMERGENTI

4 - EPIDEMIOLOGY AND GENOMIC SURVEILLANCE OF CANDIDA AURIS: EMERGING MULTIDRUG RESISTANT PATHOGEN

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Title: Epidemiology and genomic surveillance of Candida auris: Emerging multidrug resistant pathogen

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Abstract

Introduction

Prevalence of fungal infections and antifungal resistance are an increasing global public health concern, resulting in approximately 1.7 million deaths every year. Moreover, the long-term therapeutic application and prophylactic use of antifungal drugs in high-risk patients has promoted the emergence of drug-resistant fungi, including the extremely virulent Candida auris. Therefore, fungal infections have become increasingly severe and critical challenge for developing countries which are more vulnerable to its impacts. High burden of infectious diseases, poverty, weak governance and health systems, and low awareness remain major challenges in the fight against AMR leading to increased prevalence of HAIs including fungal infections, and superbugs like C. auris. National Institutes of Health Pakistan with support of CDC, launched a study to improve the early diagnosis and surveillance of C. auris. It focused on capacity building in laboratory diagnostics, IPC strategies and implementing robust learning data dashboards with provincial stakeholders.

Materials and Methods

Twelve tertiary care hospitals across country were assessed thoroughly using the LAARC tool (CDC), Fungal Assessment Tool, IPCAF, IPCOT tools (WHO). Gaps in terms of human resource, supplies, reagents, sample transport, external quality assurance and data management were addressed at all hospitals and laboratories. Phase wise capacity building on laboratory diagnostics, IPC measures, and outbreak management were conducted.

Results

In two years after the launch of program, in microbiology diagnostics all 12 sites initiated reporting fungal infections to NIH and 5 of the sites have been enabled in confirming their diagnosis independently apart from being the part of the collective reporting system. More than 5700 clinical samples processed for fungal cultures at all laboratories. 2100 various Candida species were reported out of which 21 have been C. auris. One of the site faced outbreak of C. auris (13 cases reported from May-Dec 2023), with mortality reported in 6/13 patient. Outbreak was controlled through robust engagement and implementing IPC measures. Whole genome sequencing for C. auris showed close association of strains to South Asian clade.

Discussion and Conclusion

The emergence of multidrug-resistant C. auris requires robust measures for diagnosis and management, particularly in developing countries to prevent outbreaks and adverse healthcare outcomes. Through this study a successful and sustainable model for National Fungal Disease Surveillance System (NFDSS) has been devised to enable rapid diagnosis, strengthen the epidemiology and surveillance of prevalent fungal pathogens throughout Pakistan. Patient management and safety is ensured through commitment of the national and sentinel hospitals. Adoption of these strategies will assist developing countries overcome their increased vulnerability to growing fungal infections and eventually the antimicrobial resistance.

8 - DECIPHERING HOST-PATHOGEN INTERACTION TO ERADICATE INTRACELLULAR MYCOBACTERIUM ABSCESSUS PATHOGEN

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Deciphering host-pathogen interaction to eradicate intracellular Mycobacterium abscessus pathogen.

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Introduction

Mycobacterium abscessus (Mab) is an opportunistic pathogen that infects people with cystic fibrosis (CF). Its incidence is increasing in this population, resulting in increased morbidity and mortality. The emergence of Mab as a CF pathogen can be attributed to several factors, particularly its natural resistance to numerous antibiotics. Little is known about the early stages of infections in humans, the Early Antigenic Secretion (ESX) systems seem to be involved. ESX systems are indeed highly conserved among mycobacterial species, linked to intracellular lifestyle. Mycobacterium tuberculosis (Mtb), the etiological agent of tuberculosis, encodes five Type VII secretion systems (T7SS) (ESX-1 to 5), all sharing common features: the presence of small, secreted proteins with a conserved Trp-X-Gly (WXG) motif, an ATPase, and several transmembrane proteins. Unlike Mtb, which is strictly pathogenic, Mab has only two ESX systems (ESX-3 and ESX-4). The ancestral ESX-4 has been shown to be a modulator of Mab infection pathophysiology, affecting its survival. Curiously, Mtb has evolved with non-functional ESX-4. ESX-3 role in Mab is still unknown while ESX-3 is necessary in Mtb for the uptake of iron and zinc and prevents phagosome maturation, counteracting the host’s efforts to eliminate the pathogen.

Materials and Methods

To study the role of ESX-3 and ESX-4 in host-pathogen interaction in Mab, we generated isogenic mutants by deleting esxGH or esxUT gene pairs, each encoding two effector proteins belonging to the WXG family, resulting in the knock-out (KO) of ESX-3 or ESX-4 functions. KO mutants were obtained using the recombineering system. The deleted genes will be reintroduced as a couple or as a single gene in an integrative plasmid to complement the mutants. The impact of esxGH or esxUT silencing in Mab ATCC19977 growth and in different ex vivo models of infection is being evaluated by microbiological and transcriptomic approaches.

Results

The constructs for obtaining KO mutants were transformed in Mab to investigate the role of ESX-3 and ESX-4 systems. PCR confirmed deletion of esxGH or esxUT genes in the KO mutants. Their function will be restored by re-introducing the gene under a constitutive promoter of an integrative plasmid. Parallelly, over-expressor strains were constructed by inserting an extra copy of the genes into the Mab genome. All strains constructed in this project are used to investigate different ex vivo models of infection (monolayers, TPH1, granulomas). Finally, all the strains will be studied by transcriptomic approach to assess their role in Mab.

Discussion and Conclusions

This work will elucidate the virulence of the emerging pathogen Mab.

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10 - CITROBACTER FREUNDII ST396: A GLOBAL RESERVOIR OF FOSA7.9 DETERMINANT

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Citrobacter freundii ST396: a global reservoir of fosA7.9 determinant

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Introduction: Fosfomycin (FOS) is an “old” antibiotic, yet, effective for treating infections caused by several Multi-Drug Resistant pathogens. Citrobacter freundii is considered a low-risk pathogen in clinical settings, although can act as a silent reservoir of relevant resistance genes. Aim of the present study was to make a molecular comparison between a clinical strain of FosA7.9-producing C. freundii ST396 (CFR_206 strain) and other C. freundii genomes identified worldwide, to investigate the role of ST396 as potential reservoir of fosA7.9 gene variant. Materials and Methods: On 31st January 2021, a FOS-resistant C. freundii clinical strain (CFR_206) was isolated from a rectal swab, as part of passive surveillance program at Modena Hospital, Italy. FOS MICs were assessed by agar-dilution method according to EUCAST 2023 guidelines, while FosA production was ascertained by PPF Test. Whole-genome sequencing (WGS) was conducted on CFR_206 strain through Illumina platform, and the results were compared with those of N=62 genomes available, retrieved from NCBI. Results: Based on WGS analysis, CFR_206 strain belongs to ST396 and carries beta-lactams (blaVIM-1) and fosfomycin (fosA7.9) resistance genes. All the ST396 members harbored at chromosomal level the fosA7.9 gene, located in into a 12,065-bp cassette consisting of the following genes: the HNH endonuclease gene, fosA7.9, fic, the type II endonuclease restriction gene, and the methyltransferase gene. Interestingly, 38.7% of ST396 harbored two copies of the fosA7.9 cassette. Based on the available metadata, C. freundii ST396 is an uncommon human pathogen with a restricted geographical spread in USA, UK, China, Pakistan, Germany and Australia. Moreover, C. freundii ST396, since 2015, is involved in the acquisition and spread of several clinical relevant antimicrobial resistance genes, such as blaKPC-2 and blaOXA-48. The identification of ST396 as a possible reservoir of fosA7.9 genes, furtherly restricts the number of effective antibiotics, limiting the potential use of FOS. Discussion and Conclusion: Despite the large use of FOS in empiric therapy in Italy, dedicated surveillance programs to monitor FOS resistance are lacking in Italy, especially in low-risk pathogens as C. freundii. The stabilization of FosA7.9 enzyme, together with successful antimicrobial resistance genes can seriously affect the effectiveness of antibiotics and limits drug availability.

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12 - KLEBSIELLA PNEUMONIAE ST22: A NOVEL MENACE?

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Klebsiella pneumoniae ST22: a novel menace?

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Introduction: We recently reported ST22 Klebsiella pneumoniae carrying a blaKPC-166 at Niguarda Hospital, Italy. Aim of the present work was to investigate the pan-genomic epidemiology of a global ST22 collection. Materials and Methods: N=254 genomes, retrieved from NCBI, were evaluated for resistome, virulome and K locus, using Kleborate. Plasmid replicon typing and pMLST were assessed, and the genomes analyzed with Roary 3.13.0, to infer core- and pan-genomes. Associations between gene presence/absence and phenotypic/metadata traits were calculated with Scoary. Fastbaps 1.0.6 defined clusters from the core gene alignment conditioned on the core gene tree. Results: Four major clusters (CL1-CL4) were identified by core genome phylogeny. Based on Roary, ST22 was associated with 21,550 cluster genes: 3,731 composing the core, 492 the soft-core, 1,397 the shell, and 15,930 the cloud. Clusters-related accessory genomes differences were detected: CL1 and CL4 showed a smaller gene content of 105 and 99 genes, respectively, and CL2 and CL3 an enrichment of 301 and 219 genes, respectively. ARGs against aminoglycosides (41,25%), fluoroquinolones (37,75%), sulphonamides (40,5%), trimethoprim (38,5%), beta-lactams (38,75%), tetracycline (33,25%) and carbapenems (24,25%) were detected. CL2 resulted the less represented by ARGs (average= 3,4), while CL4 carried significantly more ARGs than the CL1 and CL2 (average= 8,74; CL1 p= 0,002; CL2 p= 0,000). The four CLs shared ARGs for beta-lactams (including carbapenems) and tetracyclines. ARGs for aminoglycosides, fluoroquinolones, macrolides, phenicol, sulphonamides and trimethoprim were common in all CLs except CL2. Fosfomycin ARGs were shared by CL2 and CL4. Colistin and tigecycline ARGs were in CL4 only. A wide distribution of plasmid replicons was detected, with a predominance of IncFIB(K) (87%). The IncF group was significantly represented by three pMLST: F-:A-:B-:K- (27%), F-:A-:B-:K7 (18.1%), and F-:A-:B-:K9 (8.3%). CL1 and CL4 were mainly represented by RST F-:A-:B-, while in CLl2 and CL3 the prevalence of F-:A-:B-:K7, F-:A-:B-:K15 and F-:A-:B-:K5 was pointed out. The overall virulence score was low n = 0, in 82% of cases. The ST22 capsular type K9 showed the highest incidence (65%), predominantly in CL4 and, at lower extent, in CL1(46%)/CL3 (42%); K10, K11, K106, K147 and K169 were detected. K11 was restricted to CL1, CL3 and CL4, K147 is found in CL1 and CL4 only, while K169 seemed a CL2 prerogative. Discussion and Conclusions: These data enrich the knowledge on emerging ST22.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

13 - HIGHLY HOMOLOGOUS FLAVIVIRUS SNS1 PROTEINS AFFECT BETA-AMYLOID DEPOSITION, IMMUNOREGULATION, ANGIOGENESIS AND COAGULATOIN PROCESSES.

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HIGHLY HOMOLOGOUS FLAVIVIRUS SNS1 PROTEINS AFFECT BETA-AMYLOID DEPOSITION, IMMUNOREGULATION, ANGIOGENESIS AND COAGULATOIN PROCESSES.

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Introduction: Over the past few years, the northeastern region of Italy, particularly Veneto and Emilia-Romagna, has witnessed a notable rise in the incidence of Flavivirus infections like West Nile Virus and Dengue, attributable to the increased proliferation of infected arthropod vectors. Among the highly conserved proteins within Flaviviruses, non-structural protein 1 (NS1) stands out, being actively released by infected cells and detectable in the bloodstream. Despite their close resemblance and similar glycosylation patterns, the different extracellular forms of NS1 are associated with distinct mechanisms in Flavivirus pathogenesis, including immune modulation, endothelial dysfunction, and neuro-invasion.

Aim: This study aims to explore the potential impact of soluble NS1 derived from different Flaviviruses (DENV, WNV, YFV, JEV, and TBEV) on critical biological processes: 1. amyloid beta (Aβ) deposition, crucial in the antiviral response during central nervous system (CNS) infection; 2. induction of the immuneregulatory and angiogenetic factor Human Leukocyte Antigen (HLA)-G; and 3. alteration of coagulation.

Methods: Human glial cells T98G (ATCC CRL-1690) and human monocyte cells THP1 (ATCC TIB-202) were exposed to hexameric soluble (s)NS1 from DENV, WNV, YFV, JEV, and TBEV. Subsequently, cell supernatants and pellets were collected and subjected to analysis for protein expression, transcription levels, and activity assays related to Aβ, soluble (s)HLA-G, and key factors of the coagulation cascade (PROS1, FX, FVII, and TF).

Results: Different biological effects were observed in response to sNS1 treatments. Notably, treatment with WNV sNS1 significantly induced Aβ deposition, consistent with its known neuro-invasive properties, heightened sHLA-G secretion, and displayed a pro-coagulative role by upregulating FVII and TF expression. Conversely, exposure to DENV-1 sNS1 exhibited an opposite

trend, affirming its anticoagulant function, which corresponded with reduced sHLA-G levels. We explored the different pathways that might affect this different behavior.

Conclusion: These preliminary results confirm the different biological effects associated with these closely related viral proteins, wherein subtle sequence and glycosylation variations appear to enable Flavivirus sNS1s to elicit distinct cellular pathways, likely through interactions with specific cellular receptors.

27 - THE "ODD COUPLE": PANCREATITIS AND STRONGYLOIDES STERCORALIS

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The “odd couple”: pancreatitis and Strongyloides stercoralis

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INTRODUCTION

Strongyloides stercoralis is one of the most neglected soil-transmitted helminths, causing a clinical spectrum of disease ranging from subclinical forms in immunocompetent patients to a life-threatening infection in patients with co-morbidities, who are predisposed to hyperinfection and disseminated disease. Herein, we describe a case of strongyloidosis in an alcoholic patient with pancreatitis.

MATERIALS AND METHODS

A 49-year-old Mauritian alcoholic patient entered the emergency room of the University hospital Policlinico in Catania, complaining of pain in the upper abdomen. Laboratory tests showed an increase in inflammation markers, neutrophilia (86.5%), and elevated lipase (2024.8 U/L). Subsequent routine tests showed increasing eosinophilia (up to 22%) and a high IgE titer (933 UI/mL). Physical and computerized exams were firstly required. Three clinical stool samples, collected on alternative days, and one sputum sample were analyzed at the Parasitology Unit.

RESULTS

The TC abdomen was compatible with a picture of edematous pancreatitis and COPD, for which aerosolized cortisone was given. The parasitological stool exam, performed using a Koga agar plate, highlighted the presence of rhabditoid larvae of S. stercoralis, not found in the sputum. The patient was treated with ivermectin (single oral dose of 200 µg/kg) and discharged upon improvement of clinical conditions.

DISCUSSION AND CONCLUSIONS

Chronic alcoholism and corticosteroids assumption are important predisposing factors for severe strongyloidosis, as they seem to elevate cortisol levels, stimulating the transformation of rhabditiform larvae into filariform larvae and inducing autoinfection. Despite this, our patient did not have a high parasite load and a disseminated infection. An answer may be sought in the infection-induced production of IgE, which has been suggested to decrease the parasite load and determine acquired resistance to autoinfection. In this case, the manifestation of pancreatitis was presumably caused by

chronic alcohol consumption and not by migration of the larvae through the biliary tract, although this occurrence has been reported as a rare complication. This case aims to highlight the importance of awareness and suspicion of strongyloidosis in immunosuppressed subjects or those with risk factors, such as alcoholism, as well as the importance of an early diagnosis that allows a rapid and targeted treatment, avoiding the possibility of dissemination in predisposed subjects.

30 - TO THE UNKNOWN: A STEP FORWARD IN THE GENOME ANALYSIS OF TOSCANA VIRUS THROUGH A NOVEL AMPLICON-BASED WHOLE GENOME SEQUENCING APPROACH

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To the Unknown: A Step Forward in the Genome Analysis of Toscana Virus Through a Novel Amplicon-Based Whole Genome Sequencing Approach

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Introduction

Toscana virus (TOSV, Phlebovirus genus) is a neurotropic sandfly-transmitted virus whose distribution encompasses various countries within the Mediterranean basin. TOSV infection remains a non-notifiable disease in Europe with no official surveillance plans in place despite being considered a significant public health concern in Southern Mediterranean countries. In order to mitigate the impact of this virus on human health, it is crucial to gain a deeper understanding beyond the anecdotal and fragmented evidence that currently exists. The aim of this study is to develop an amplicon-based whole genome sequencing method suitable for the genomic characterization of TOSV.

Materials and Methods

Primers were designed on TOSV lineage A S, M and L segments reference sequences (GenBank: GCA_031497085.1) using Primal Scheme web tool to generate 400 bp tiled amplicons. To guarantee coverage and sensitivity, a multi-sequence alignment of GenBank-available complete sequences was used to identify mismatches and introduce degenerations in relevant ambiguous sites. Two-pool multiplex amplicon-based library preparation was carried out with Illumina Microbial Amplicon Prep (iMAP) and custom designed primers. Two viral isolates on Vero E6 cells and three clinical samples of patients presenting with meningitis were sequenced. De novo assembly was performed with BaseSpace DRAGEN Targeted Microbial (version 1.3.1).

Results

A set of 45 oligonucleotide primer pairs (26 for segment L, 13 for segment M and 6 for segment S) that amplify overlapping segments spanning TOSV genome was generated. The primers sets were subsequently compared to an alignment of 8 sequences for segment L, 16 for segment M and 32 for segment S to introduce degenerations. Genome sequencing yielded a mean 98.2% coverage for high-viral-titre propagates on Vero E6 cell culture. Two human samples (1 urine and 1 cerebrospinal fluid)

with Ct (Cycle threshold) values of 32 and 33 yielded to sequences with coverage of 91.3% and 80.3%. Another urine sample with Ct = 35 yielded to 47% of mean coverage.

Discussion and Conclusions

Our study, albeit with preliminary and limited but very promising results for high-viral-load propagates and low-viral-load samples, proposes a new whole genome amplicon-based sequencing method for genomic characterization of TOSV one of the most neglected and least monitored arboviruses. Alongside an increasingly necessary systematic epidemiological monitoring of TOSV infections, WGS may be instrumental to unravel its genomic makeup and to gain insights into its biology and transmission dynamics. This knowledge can inform the development of new diagnostics, therapeutics, and preventive measures, particularly in TOSV high risk regions.

31 - PRESSURIZE TO IMMUNIZE: HARNESSING HIGH HYDROSTATIC PRESSURE FOR WHOLE INACTIVATED VIRUS VACCINES

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Pressurize to Immunize: Harnessing High Hydrostatic Pressure for Whole Inactivated Virus Vaccines

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Introduction

Viral inactivation constitutes the basis of many immunoprophylaxis strategies, alongside subunit and, more recently, nucleic-acid-based vaccines. The growing and continuous demand for vaccine doses has stimulated research, boosting the development of new viral inactivation techniques, like high hydrostatic pressure inactivation (HHP), which are already used in the food industry to control microbial contaminations. Studying the efficacy of this method to inactivate viruses and evaluating the structure and antigenicity retention of major immunodominant sites, together with their immunogenicity, may offer new possibilities.

Materials and Methods

HHP-mediated inactivation of SARS-CoV-2 B.1 and BQ.1.1 lineages ultracentrifugation-purified viral isolates was carried out with a high-pressure system (Avure Technologies Inc.). Three different pressures, each maintained for 5 minutes, were tested: 400, 500 and 600 MPa. Virus infectivity reduction and complete inactivation were assessed on Vero E6 cell culture. Virion ultrastructure and its antigenicity were evaluated by negative staining transmission electron microscopy and western blot. Immunogenicity was measured in a murine model to assess the induction of B- and T-cell-mediated responses (ELISA, seroneutralisation and T-SPOT assays).

Results

Both variants were completely inactivated at 500 and 600 MPa, while virus isolates treated at 400 MPa retained partial infectivity. Even at the highest tested pressure, the viral particles were morphologically identifiable. Inactivation from 500 MPa nonetheless resulted in alterations of the outer surface, which

appeared smoother due to spikes (S) damage. In agreement with TEM observation, while the ability of the spikes to bind antibodies in western blot was reduced compared with the non-HHP-inactivated control, other structural proteins i.e., nucleocapsid (N) and membrane (M), were not damaged. Inactivated viruses were thus capable to induce both an antibody and cell-mediated response in the animal model.

Discussion and Conclusions

Preliminary results show HHP efficacy in producing viral inactivates that can efficiently be used as immunogens in murine animal models. The process has so far been validated against SARS-CoV-2, but can universally be used for other emerging and re-emerging viruses with a high impact on human and animal health. The same inactivation process is currently being evaluated for the inactivation of other viruses with high health impact, with promising pilot data. The development of a HHP viral inactivation method, allowing the production of large quantities of inactivated viral suspension, might represent an important turning point with a high intrinsic potential of clinical translation in the immunoprophylactic field for the development of new low-cost (~ 5€/litre) thermostable vaccines not requiring a cold chain for distribution and suitable for use also in LMICs (Low- and Middle-Income Countries).

59 - EXPLORING THE ANTI-POXVIRUSACTIVITY OF NOVEL BENZIMIDAZOLE DERIVATIVES AS ENTRY INHIBITORS

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Exploring the anti-PoxvirusActivity of Novel benzimidazole derivatives as entry Inhibitors

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Abstract

Introduction:Infectious diseases remain the leading cause of morbidity and mortality in humans and livestock worldwide, resulting in significant health costs.

Four decades after the eradication of smallpox, poxviruses continue to threaten human and animal health. Vaccinia virus (VACV) was used as the vaccine that successfully eradicated smallpox and is a prototypic member of the poxvirus family.

Monkeypox (MPXV)is considered the most significant orthopoxvirus infection to affect humans since the eradication of smallpox. MPXV is found in wildlife (small mammals) in several Central and West African countries. Currently, the largest known MPOX epidemic is spreading worldwide, with more than 95,000 (CDC) cases to date, mostly in Europe and North America, showing a higher number of human cases and greater human-to-human transmission than previously documented. These increased cases and burdens are likely driven by decreasing global immunity. In this scenario, there is an urgent need to better understand this disease in humans and animals and develop a specific drug to treat the infection.

Here wedescribe new benzimidazole derivatives tested against representatives of the Orthopoxvirus genus, Vaccinia Virus (VV), closely related to variola virus and MPXV.

Methods: The cytotoxicity, the antiviral activity, and the study of mechanisms of action of the most interesting compounds were determined with a combination of cell-based and molecular experimental techniques.

Results: In our research, newand variously substituted benzimidazolesendowed by interesting EC50values and cytotoxic profile in the high micromolar range were not virucidal but active in the early phases of the viral cycle.

Conclusion: Our work describes interesting benzimidazole derivatives that should be further investigated for inhibiting poxvirus entry.

64 - HAZARA VIRUS ADAPTATION TO HYALOMMA TICK CELLS IS ASSOCIATED WITH MUTATIONS IN GENES ENCODING POLYMERASE AND GLYCOPROTEIN PRECURSOR

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Hazara virus adaptation to Hyalomma tick cells is associated with mutations in genes encoding polymerase and glycoprotein precursor

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Introduction

Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a tick-borne pathogen that presents a serious threat to human health, causing a severe hemorrhagic fever with high mortality rate. Ticks of the genus Hyalomma, are the principal vectors of CCHFV, harboring the virus persistently and acting as its natural reservoir. However, due to biosafety concerns, limited knowledge is available regarding the virus-vector interaction. Cycling between hosts likely contributes to the virus's evolutionary flexibility, however cross-species transmission imposes selective constraints, influencing viral adaptation. This study explores the evolutionary progression of Hazara virus (HAZV), used as a model for CCHFV, across different host cell lines, aiming to elucidate how pressure within the host environment drives viral adaptation and impacts infectivity.

Materials and Methods

We used HAZV grown in human SW13 cells to infect the Hyalomma tick cell line HAE/CTVM8. On days 30, 60 and 90 we collected samples and passaged virus onto new tick and SW13 cells, evaluated after a further 30 and 6 days respectively. Genotypic and phenotypic changes were assessed, respectively, by NGS sequencing of cell pellets and supernate and by evaluating viral infection in tick and SW13 cells.

Results

Through analysis of HAZV propagation in HAE/CTVM8 cells, we determined the emergence of mutations within all three viral genome segments. Notably, passage of HAZV within this cell line appears to lead to greater stabilization of two mutations, one synonymous and one nonsynonymous, within the gene encoding the RdRp and one nonsynonymous mutation in the glycoprotein precursor (GPC). HAZV adapted to tick cells showed a host-dependent effect on viral infection efficiency, with higher infectivity in HAE/CTVM8 compared to mammalian cells. Conversely HAZV from SW13, used as a control, showed the opposite trend.

Discussion and Conclusions

Mutations appeared in HAZV during the first 30 days and increased slowly up to 30-40% by day 90. With subsequent passage in tick cells, mutations in the RdRp and GPC increased from 15-30% to 60-80% and 20% to 50% respectively, suggesting a host-driven adaptation. Viral replication kinetics indicated that tick-adapted HAZV replicates better in tick cells, suggesting a potential role of the selected mutations in viral adaptation to the invertebrate host. Although genetic changes in passaged HAZV were minimal they seemed to lead to increased relative fitness and replicative ability of the virus in the homologous HAE/CTVM8 cell line. Further experiments will be performed after isolation of mutated viruses.

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82 - IDENTIFICATION OF EARLY INFLAMMATORY MEDIATORS INDUCED BY SARS-COV-2 AS PROGNOSTIC MARKERS OF DISEASE SEVERITY: A PILOT STUDY

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Identification of early inflammatory mediators induced by SARS-CoV-2 as prognostic markers of disease severity: a pilot study

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Introduction: Clinical studies indicate that patients infected with Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus develop hyperinflammation, which correlates with worsened disease progression and increased mortality. The SARS-CoV-2/Coronavirus disease 2019 (COVID-19)-dependent inflammation is thought to occur mainly via innate immune activation and increased cytokines production. The purpose of this pilot study is to define predictive biomarkers of pathogenic inflammation prognostic of severe disease.

Materials and Methods: A retrospective cross-sectional cohort study will be conducted on 103 serum samples from patients hospitalized with SARS-CoV-2 infection during the 2020 pandemic at the Umberto I Hospital (Rome, Italy). Sample selection criteria were applied to exclude patients with concomitant respiratory and bloodstream infections due to other pathogens. Seven cytokines and chemokines involved in the inflammatory response will be quantified by multiparametric ELISA tests.

Results: The study sample consisted of sera collected between 12/02/2020 and 26/04/2020 from hospitalized patients with a COVID-19 confirmed diagnosis. Mean age of patients was 62 years (range 25-95) and 41,7% were women. Mean length of hospital stay was 19 days (range 1-86). Information on the potential clinical inflammatory biomarkers C-reactive protein, D-dimer, LDH, albumin, was available. Out of the total study sample, 50 and 38 patients were receiving immunosuppressant and antiviral drugs, respectively. Seven severe cases required intensive care unit admission and 12 deaths

were reported. Multiparametric analysis to quantify serum levels of IL-6, IL-1beta, TNF-alpha, IL-10, IP-10, CCL3, IL-8 is in progress.

Discussion and Conclusions: The retrospective cohort study has been conceived to facilitate the identification of predictive biomarkers of COVID-19 severity. The study will provide crucial information on the expression of selected inflammatory mediators in a cohort of patients characterized by a broad spectrum of COVID-19 severity. The results obtained could be useful for the set-up of novel and larger cohort studies on serum samples collected from patients hospitalised with severe respiratory disease infection.

86 - SARS-COV-2 INTRA- AND INTER-HOST EVOLUTION IN COVID-19 HOSPITALISED PATIENTS WITH PERSISTENT INFECTION

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SARS-CoV-2 intra- and inter-host evolution in COVID-19 hospitalised patients with persistent infection

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Introduction: SARS-CoV-2 persistent replication during long-term infection could be the most plausible hypothesis to explain the origin of Variants of Concern (VOCs). Indeed, an accelerated intra-host evolution and heightened viral genetic diversity were observed, with lineage-defining mutations closely resembling those found in VOCs. In this retrospective longitudinal study, we delineated intra-

and inter-host genetic diversity and virus evolution during SARS-CoV-2 persistent infection. Additionally, the intra-host analysis of minor variants was performed. Materials and Methods: Viral isolates from eight patients with persistent infection attending at Intensive Care Unit (ICU) and Infectious Diseases Unit were included. Whole Genome Sequencing (WGS) and phylogenetic analysis on twenty-seven nasopharyngeal swabs collected at different time points (up to 90 days) were performed. Variants frequency was set to ≥5%. Results: Phylogenomic reconstruction showed that the predominant variant among our persistently SARS-CoV-2 infected patients was Omicron BA.5, followed by Omicron BA.1 and BA.2 sub-lineages. The Bayesian inference revealed a rapid intra-host diversification and emergence of new genetically divergent isolates during viral persistence. At the same time, the PCoA analysis showed a host-based genomic structuring among antigenically divergent variants, that might reflect the positive effect of containment practices within the critical Hospital areas. Interestingly, intra-host divergent variants exhibited the same combination of amino acidic substitutions across the entire genome, particularly in the Spike glycoprotein, able to increase viral transmissibility (K417N, S477N, N501Y and Q498R), to enhance infectivity (R346T, S373P, R408S, T478K, Q498R, Y505H, D614G, H655Y, N679K and P681H), to determine host immune escape (S371L, S375F, T376A, K417N, and K444T/R) and to display partial or complete resistance to treatments (G339D, R346K/T, S371F/L, S375F, T376A, D405N, N440K, G446S, N460K, E484A, F486V, Q493R, G496S and Q498R). Low-frequencies mutations analysis revealed the co-existence of minor variants within the same host. Discussion and Conclusions: Since the events leading to the spread of divergent SARS-CoV-2 lineages during the pandemic remain unclear, our results are consistent with the hypothesis that chronic SARS-CoV-2 persistent patients could represent a “reservoir” of viral adaptation. The generation of intra-host “quasispecies” might cause a jump in the otherwise clock-like evolutionary rate of SARS-CoV-2. In the Omicron era, a pro-active genomic surveillance of persistent SARS-CoV-2 infected patients is recommended to characterized genetically divergent lineages before their diffusion.

124 - SEQUENCING OF SARS-COV-2 VARIANTS FROM FAECAL SAMPLES OF DIARRHOEIC PATIENTS WITH CONCOMITANT POSITIVITY FOR PARASITIC INFECTIONS

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Sequencing of SARS-CoV-2 variants from faecal samples of diarrhoeic patients with concomitant positivity for parasitic infections

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Introduction: In the COVID-19 disease, diarrhoea, could be one of the initial presenting symptoms, with a susceptibility and severity of infection that vary significantly across patients. Gastrointestinal protozoan parasites (GPPs) contribute significantly to the burden of illness worldwide with diarrhoea being the most common among associated gastrointestinal symptoms. Although several authors have speculated that some parasites could exacerbate the severity of viral infections, the link between parasitic and SARS-CoV-2 infection remain still unclear. In a previous work, we found a positive association between the classic GPPs (i.e. Blastocystis sp.) and SARS-CoV-2 positivity in patients with diarrhoea. Therefore, in this study we adopted the SARS-CoV-2 whole genome sequencing approach directly from faeces of patients with concomitant presence of GPPs trying to better understand a potential connection between COVID-19 cases and parasitic infections.

Material and methods: Over the period 2022-2024, 20 individual non-duplicated faecal samples from 1181 patients admitted to “Policlinico Riuniti” (Foggia, Italy) for several admission diagnosis and that presented diarrhoea were found positive for both SARS-CoV-2 and GPPs by AllplexTM SARS-CoV-2 and GI Parasite Assay (Seegene, Korea), respectively. Then, they were subjected to next generation sequencing amplification by CleanPlexTM SARS-CoV-2 (Illumina Platform). A potential relationship between SARS-CoV-2 variants and one or more pathogenetic subtypes/genotypes/assemblages GPPs was also analysed.

Results: Out of 20 investigated faecal samples, 11 were successfully sequenced and two recombinants, eight omicrons, and one delta SARS-CoV-2 variants were identified. Omicron variants were found in four patients co-infected with Blastocystis ST2-ST4, two patients co-infected with Giardia duodenalis Assemblage A, one patient co-infected with Dientamoeba fragilis genotype I and

one patient co-infected with Blastocystis ST2/D. fragilis. Recombinants virus were found in one patient co-infected with Blastocystis ST3 and one co-infected with G. duodenalis Assemblage A. Delta variant was detected in one patient co-infected with Blastocystis ST2/D. fragilis.

Discussion and Conclusions: In this work we were able to sequence the SARS-CoV-2 genome directly from faeces of patients with concomitant GPPs positivity. This finding further supports the hypothesis of a significant contribution of SARS-CoV-2 in exacerbating gastrointestinal symptoms associated with GPPs infection. Further investigations with larger samples size are needed to better clarify this finding.

132 - TRACING THE ORIGIN, SPREAD AND MOLECULAR EVOLUTION OF DENGUE TYPE 1 CASES OCCURRED IN NORTHERN ITALY IN 2023

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Title: Tracing the origin, spread and molecular evolution of Dengue type 1 cases occurred in Northern Italy in 2023.

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Background: Dengue virus (DENV) is a mosquito-borne Flavivirus that is endemic in many tropical and sub-tropical countries where the transmission vectors Aedes spp. mosquitoes reside. Dengue disease is caused by genetically distinct serotypes, DENV-1-4. Here, we present data on DENV-1, isolated from 13 patients during an autochthonous outbreak in Lombardy Region (Northern Italy) in August 2023 that were analyzed by whole genome sequencing. Adult mosquitoes of the neighboring area were also analyzed and sequenced. We also included in the analysis three cases of imported DENV-1 from Italian people living in Lombardy who traveled between July and August 2023 in Asian countries.

Methods: Metagenomic approach was used to sequence the samples and recover the whole genome of DENV-1. INSaFLU pipeline was used to construct consensus sequences. NCBI BLASTn program was exploited to perform multiple sequence alignments of DENV-1 sequences with Standard databases (Query Coverage and Identity Percentage from 96% to 99%). A total of 540 sequences were collected to perform phylogenetic analysis. Genotype analysis was performed with the Genome Detective tool. The maximum likelihood tree was generated with IQ-TREE v1.6.8. The emergence and the dynamics of DENV-1 in Lombardy were inferred on a non-redundant dataset of representative sequences by Bayesian methods (BEAST v1.10.4).

Results: The final dataset included 540 sequences from Europe (3%), Asia (5%), Northern America (32%) and Southern America (60%). Time of sequencing spread from 1977 to 2023 with most abundant samples in 2005-2009 (32%) and 2020-2023 (35%). The 14 autochthonous Italian strains clustered with Southern America strains (Peruvian and Brazilian) collected in the period 2020 to 2023, with an average nucleotide identity of 99.47% (range: 99.0–99.7) between DENV-1 strains of genotype V subtype D. The 3 imported Italian strains clustered with Asian strains (China and Viet Nam) collected in the period 2015-2019 and 2020-2023, with an average nucleotide identity of 97.76% (range: 95.9–99.9) between DENV-1 strains of genotype I subtype K and E. To determine which lineages circulated in Italy and were associated with the outbreak, we resampled the sequences by countries with large numbers of strains based on the amount of sequences available for the countries that were less sampled. As a result, we obtained a non-redundant representative dataset (n=252) preserving genomes of interest as well as minimizing the loss of genetic variation. The Bayesian analysis

estimated a mean evolutionary substitution rate of 7.861x10-4 subs/site/years.The time to the most recent common ancestor estimate was 1943.

Conclusion: The results aim to provide better insights in the spread dynamics of DENV-1 Lombardy outbreak and to advocate for the need for molecular surveillance to guide public health interventions.

136 - ASSESSING THE CANDIDA AURIS OUTBREAK IN A REFERRAL INTENSIVE CARE UNIT USING THE IR BIOTYPER

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Assessing the Candida auris outbreak in a referral intensive care unit using the IR Biotyper

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Introduction

Candida auris is an emergent pathogen recently reported in the WHO fungal priority list. It was firstly isolated in a Japanese patient in 2009 but evidence of its presence in hospital settings date back to 1996 in South Korean patients. Currently, C. auris is spreading worldwide: it has been detected in more than 30 countries and it has been linked to outbreaks in healthcare centers. This pathogen shows several virulence factors, such as thermotolerance, osmotolerance, and biofilm production. These yeast's features contribute to the long-term persistence and survival on biotic and abiotic surfaces. Following epidemic clusters in Northern Italy, including Piedmont, a retrospective epidemiological analysis was carried out to investigate the C. auris nosocomial outbreak occurred in the Intensive Care Unit of the University Hospital Città della Salute e della Scienza di Torino, Italy.

Materials and Methods

From December 2021 to January 2023, 40 C. auris strains isolated from different sites (e.g. groin swabs, urinary catheters, bronchoalveolar lavages, sputum and blood cultures) were considered. All strains were cultured on Sabouraud Agar and incubated at 37°C for 24 h. The isolated colonies were identified by using a Bruker microflex MALDI-TOF, and then tested with the Fourier-Transform Infrared spectroscopy-based IR Biotyper (Bruker) system according to the manufacturer instructions.

Quintuplicate analyses of every strain were conducted in three separate experiments. OPUS v.8.2 and IR Biotyper Client v.4.0 software (Bruker) were used to analyse the spectra. Two external strains were used as a control for clustering. Metadata on clinical and epidemiological characteristics, length of hospital stay, patients' room and bed were gathered.

Results

A dendrogram and a 2D/3D scatter plot were obtained, respectively, from the hierarchical cluster algorithm and Linear Discriminant Analysis. These show three distinct clusters: the alfa-cluster, which consisted of 36 strains; the beta-cluster, which contained 4 strains; and the gamma-cluster, which was identified by the strains external to the clusters. The metadata did not define a clear epidemiological link among C. auris strains involved in the clusters.

Discussion and Conclusions

The use of the IR Biotyper made it possible to gather crucial phylogenetic information regarding the connections between the C. auris strains involved in the local outbreak. The external gamma-cluster strengthened the phylogenetic distance between the two observed hospital clusters. IR Biotyper could be considered a promising tool for real-time hospital infection control procedures because of its simple workflow, quick turnaround time, easy-to-use interface, and low price. It also allows for prompt and reliable microbial typing.

168 - HYLIN-A1 ANTIVIRAL POTENTIAL AGAINST WEST-NILE VIRUS INFECTION

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Hylin-a1 antiviral potential against West-Nile virus infection

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Introduction. Given the emergence of the coronavirus disease 2019 (COVID-19), zoonoses have raised in the spotlight of the scientific community. Animals have a pivotal role not only for this infection, but also for many other recent emerging and re-emerging viral diseases, where they may represent both intermediate hosts and/or vectors for zoonoses diffusion. Today, roughly two-thirds of human infections are derived from animal origins; therefore, the search for new broad-spectrum antiviral molecules is mandatory to prevent, control and eradicate future epidemic outbreaks.

In this scenario, AMPs with antiviral effect, also known as AVPs, have been already reported as potent inhibitors of the viral infection by affecting different stages of virus lifecycle. We recently reported the strong potential of Hylin-a1 peptide as a pan-inhibitor of several RNA viruses responsible for respiratory infections, including the coronaviruses HCoV-229E and SARS-CoV-2, measles virus, human parainfluenza virus type 3 and influenza virus H1N1. In the present study, our aim was to deepen Hylin-a1 spectrum of action to the flavivirus West-Nile. Materials and methods. AVPs were synthesized using the solid-phase Fmoc chemistry method, followed by purification by reversed-phase HPLC. The toxicity was determined via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the hemolysis was investigated on human erythrocytes, identifying the 50% cytotoxic concentration (CC50) and the concentration causing 50% hemolysis of red blood cells (HD50), respectively. Different time of addition assays were performed to evaluate in which part of the

viral lifecycle AVPs could be involved and results were confirmed by Real-Time PCR. In addition, the interaction of Hylin-a1 with membrane was further investigated. Results. The antiviral activity was investigated indicating that Hylin-a1 was able to reduce West-Nile virus (WNV) replication in a dose-dependent manner. Results were confirmed by Real-Time PCR, observing very low expression levels of viral genes at the highest concentrations of the peptide. In detail, the main target of Hylin-a1 was the WNV envelope, as also observed by the interaction with liposomes mimicking the viral membrane. Probably, the antiviral effect of Hylin-a1 was due to the interaction with the negative-charged viral membrane, which probably caused a physical disruption of the lipid bilayer of the viral envelope (virolysis). Discussion and conclusions. Considering AVPs huge therapeutic potential, further experiments are required to confirm their mechanism of action and to increase their stability by modifying the native sequence.

178 - IMPACT OF CIRCULATING AND BLOOD ANELLOVIROMA IN IMMUNITY AND FRAILTY SYNDROME

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Topic: EMERGING AND RE-EMERGING VIRUSES

Title: Impact of circulating and blood anelloviroma in immunity and frailty syndrome

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iNTRODUCTION: The most abundant components of the human virome, the Anelloviridae

family, could provide a simple, accurate, universal, and non-invasive test to precisely assess the immune system responsiveness, particularly in geriatric clinical settings (e.g. frail elderly subjects). Circumstantial evidence has recently reported that immunity strictly modulates the replication of one of these viruses Torquetenovirus (TTV) and that TTV viremia tends to be higher in patients with immune system dysfunction compared to healthy controls. TTV load also increases with aging and is associated with mortality in the elderly. The present project intends to approach this issue by investigation of the virome with a particolar focus on anelloviruses and TTV. Viral load, virome complexity will be studied in 300 biological samples derived from large-scale integrated projects focused on biomarkers of aging and frailty. The subjects are divided into cohorts with different functional immune responses, classified from 1 to 3: a) healthy adult volunteers not taking drugs and with a fully effective immune response (Cohort 1A); b) healthy adult volunteers with a reduced but still preserved immune response (Cohort1B); c) elderly volunteers with evidence of an altered immune response and/or immunosenescence (Cohort1C).

The expected aims are: i) To significantly contribute to the validation of TTV or other components of the virome as innovative biomarkers of immunity and for the personalization and optimization of the management of geriatric frail subjects; ii) to improve our knowledge on the still unexplored relationship between age-related changes in the virome, chronic inflammation and frailty.

MATHERIAL AND METHODS: The study was performed on human blood samples. Viral DNA will be extracted from 200 µl of blood samples using QIAamp DNA Blood mini kit (Qiagen). The following methodologies will be used: a) UTR rtPCR for TTV quantification in blood samples. TTV presence and

loads will be determined by rtPCR, designed on a highly conserved segment of the 5-UTR of the viral genome using 5 µl nuclease TaqMan technology; b) Next generation sequencing (NGS) for genetic TTV-virome characterization were performed on TTV positive samples. Rolling circle amplification (RCA) products will be amplified by using an inverse PCR with primers designed on the highly conserved UTR of the TTV genome. The resulting PCR products will be used for constructing the library for Illumina sequencing (MiSeq 2×250 bases). Translated sequence reads showing similarity to TTV sequences will be identified using BLASTx;

RESULTS: The results of viremia in the different sample cohorts highlight that viremia correlates positively with age in Cohort 1($r=0,50$, $p<0,0001$).

The preliminary results of the anellovirome in the 40 samples processed highlighted the presence of TTV species in 2 samples, both TTV 29 species, furthermore the presence of TTMV was highlighted in 9 samples respectively: TTMV 1,5,6,7,11, 16,17,38.

DISCUSSION AND CONCLUSION: TTV DNA load and TTV species may be associated with age, clinical data, laboratori parameters, frailty, cognitive decline, functional status (Cohorts 1, 2, 3) and mortality (Cohorts 2, 3). At first, the association of Anellovirome with the immune status will be analyzed in Cohort 1. However, further investigations into the annelloviroma are ongoing.

180 - HOST DEFENCE PEPTIDES AS PROMISING STRATEGY AGAINST MULTIDRUG RESISTANT KLEBSIELLA PNEUMONIAE

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HOST DEFENCE PEPTIDES AS PROMISING STRATEGY AGAINST MULTIDRUG RESISTANT KLEBSIELLA PNEUMONIAE

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Introduction. The continuous outbreak of drug-resistant bacterial infections imposes the need to search for new drug candidates. Klebsiella pneumoniae (K. pneumoniae) infections are currently widespread in hospitals and result particularly dangerous to human health. In recent year, an increase of K. pneumoniae carbapenemase (KPC)-producing, has been observed determining respiratory and urinary tract affections. Antimicrobial peptides (AMPs) have been used as alternatives to conventional antibiotics. In this study, three peptides named pantinin-1, pantinin-2 and pantinin-3, derived from the scorpion venom of Pandinus imperator, have been studied for their antibacterial activity, focusing in depth on the action against K. pneumoniae and examining the regulation of virulence factors. Materials and Methods. Cytotoxicity of peptides was evaluated on the HaCaT cell line (human keratinocytes) with MTT method. Antibacterial activity was performed against K. pneumoniae ATCC 10031 and several KPC-producing clinical isolates through the liquid microdilution assay to assess the minimum inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). 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 effect of peptides on the gene expression of virulence factors of K. pneumoniae was evaluated through Real-Time PCR. Results. MTT test revealed that the safe concentration was 50 µg/mL for pantinin-1 and pantinin-2, while it was 25 µg/mL for pantinin-3.

All peptides had a remarkable antibacterial action against both Gram-positive and Gram-negative bacteria in a range of concentrations from 50 µg/mL to 6 µg/mL. Among Gram-negative ones, the most noticeable effect was recorded against K. pneumoniae. Pantinin-1 and 2 had a bactericidal effect already after 1h, while pantinin-3 showed a bacteriostatic action. MIC value for pantinin-1 was 6 µg/mL, lower than pantinin-2 (12 µg/mL), which in turn was lower than pantinin-3 (25 µg/mL). The assessment has also been extended to KPC-producing strains, which are β-lactamases capable of hydrolyzing carbapenems. Also in this case, the results were noteworthy with MIC recorded in a range from 50 µg/mL to 25 µg/mL. Finally, the expression of genes encoding virulence factors such as siderophores, piles, fimbria and capsular polysaccharides, has been evaluated, evidencing a a reduction in gene expression of about 40% after treatment, suggesting that peptides were capable of reducing inflammation. Discussion and Conclusions. Overall, our study indicates pantinins as a promising new therapeutic weapon against multidrug resistant bacterial infections.

183 - MONKEYPOX VIRUS REPLICATION IN THE LOW FEMALE GENITAL TRACT: HOST-VIRUS INTERPLAY AT THE VAGINAL AND ECTOCERVICAL LEVEL

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Monkeypox virus replication in the low female genital tract: host-virus interplay at the vaginal and ectocervical level

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Introduction: the recent Mpox outbreak showed a predominant transmission upon sexual contact in men having sex with men, and monkeypox virus (MPXV) was repeatedly detected in semen. Female patients accounted for 1% to >30% of cases in Mpox outbreaks, and vaginal lesions, vertical transmission, and an increased risk of miscarriage in infected women have been reported. This study aimed to investigate the susceptibility of cells from the low female genital tract (LFGT) to MPXV infection, the influence of sex hormones on viral replication, and the host-virus interplay at the vaginal and cervical levels. Materials and Methods: human immortalized epithelial vaginal (VK-2) and ectocervical (Ect1) cells were exposed to MPXV clade IIb for 2, 24, 48, and 72h. Viral replication was assessed by MPXV DNA PCR and titration of released viral particles. The influence of beta-estradiol or progesterone pretreatment at levels measured during follicular, luteal phase, or pregnancy on MPXV replication was investigated. The impact of viral infection on cellular gene expression was determined by RNAseq. ELISA was used for the determination of MMP1 content in cell supernatants. Results: efficient viral replication in VK-2 and Ect-1 cells was observed, as confirmed by the significant increase in MPXV-DNA and released infectious particles. The genital epithelial structure was disrupted by MPXV in a dose and time-dependent manner. The pretreatment with beta-estradiol (0.1, 1, 10 nM) and progesterone (1, 10, 100 nM) determined a slight decrease in the shedding of MPXV infectious particles from ectocervical cells. At 48 h, the RNAseq analysis of MPXV infected vs uninfected (n.i.) cells showed 216 differentially expressed genes (DEGs) in VK-2 and 11 DEGs in Ect-1 cells, with nine common upmodulated genes involved in protein folding (HSPA6), cell chemotaxis and migration (CXCL3, ARC), inflammation and lymphoproliferation (IL11, IL1RL1, MMP1), cell adhesion, and tissue remodeling (IGFN1, MMP1). In vaginal cells, the GO BP analysis revealed enrichment for response to unfolded protein and stress signaling, regulation of the MAPK cascade, and myeloid cell differentiation. The transcriptional up-modulation of MMP1 corresponded to an increased release of this protein in the supernatant of 72h infected VK-2 (3-fold vs n.i.; p=0.06) and Ect1 (vs n.i. 4-fold;

p=0.01) cells. Discussion and Conclusions: overall, the results demonstrated the MPXV-mediated modulation of tissue-specific and common cellular pathways in the LFGT. The dysregulation of MMP1 and IL11 has been involved in adverse birth outcomes. Further investigations are needed to dissect the pathogenetic mechanisms of MPXV in the female genital tract and reveal the impact of MPXV infection on women's sexual and reproductive health.

198 - PARVOVIRUS B19 EPIDEMIOLOGY IN THE METROPOLITAN AREA OF BOLOGNA: PRE-PANDEMIC, PANDEMIC AND POST-PANDEMIC TRENDS

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Parvovirus B19 epidemiology in the metropolitan area of Bologna: pre-pandemic, pandemic and post-pandemic trends

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INTRODUCTION

B19V infections occur according to a seasonal cycle with periodical epidemics of varying magnitude occurring during spring. During the COVID pandemics the incidence of B19V infection showed a sharp decrease, implying alert to an increase in incidence in subsequent years. The aim of this study is to assess the incidence of B19V infection over the last 10 years through the analysis of the diagnostic activity performed at the Microbiology Unit IRCCS Sant'Orsola Hospital, Bologna, Italy.

MATERIAL AND METHODS

From 2014 to April 2024, 20459 consecutive serum samples were tested for the presence of parvovirus B19 IgG/IgM antibodies (LIAISON® Biotrin Parvovirus B19 IgG, IgM - DiaSorin) and 3220 serum samples were tested for the presence of parvovirus B19V DNA (Parvovirus B19 ELITe MGB® Kit - ELITechGroup). Patients were considered acutely infected with B19V whether IgM antibodies and/or B19V DNA load >1000 copies/ml were detected.

RESULTS

B19V IgG antibodies were detected in 11014 samples (53.8%), IgM antibodies in 632 (3.1%) and B19V DNA was detected in 472 samples (14.7%).

In the 10-year retrospective analysis, 583 patients with B19V infection were identified. During the pre-pandemic period (Sep 2014-Dic 2019) 403 cases of B19V infection were detected out of 11663 patients (incidence 3.5%), whereas during the pandemic period (Jan 2020-Dic 2022) 42 cases were detected out of 5507 patients (incidence 0.8%). Subsequently, in the post-pandemic period (2023) 25 cases were detected out of 1931 patients (incidence 1.3%). Finally, from January to April 2024 a massive circulation of the virus was reported: 114 cases of B19V infections out of 963 patients screened were detected (incidence 11.8%). A statistically significant increase in B19V infections was observed from January 2023 onwards and, to a greater extent, from January 2024.

CONCLUSIONS

Our data indicate that the normal circulation of B19V, alternating between low and high incidence years, has been substantially affected in the COVID era, confirming that public health measures taken to limit the spread of SARS-CoV-2 led to a drastic reduction of B19V circulation. The subsequent rise in incidence, the highest reported in recent years, can be attributed to the larger population fraction become susceptible in the intervening years. Active surveillance for potential B19V outbreaks is warranted, confirming the importance of a prompt and accurate diagnosis by simultaneous detection of antibodies and viral DNA, especially in individuals with severe symptoms such as acute anemia or heart failure.

212 - GENETIC DIVERSITY OF NDM-PRODUCING KLEBSIELLA PNEUMONIAE CIRCULATING IN MARCHE REGIONAL HOSPITAL SINCE 2021

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Genetic diversity of NDM-producing Klebsiella pneumoniae circulating in Marche regional hospital since 2021

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Introduction: Multi drug resistant Klebsiella pneumoniae (Kp) is one of the main responsible of healthcare associated infections and its high resistance to carbapenems makes them difficult to treat. The most frequent Kp carbapenemase is blaKPC with all its variants but in the last years New Delhi metallo-beta-lactamase (NDM) detection is increasing. At the end of 2021 the first NDM producing Kp (Kp-NDM) was isolated at "Ospedali Riuniti" of Ancona, Italy, and from that detection other 16 NDM-Kp positive samples were collected up to September 2023.

Material and methods: 17 Kp-NDM were collected from different sources from patients recovered at Marche regional hospital between November 2021 and September 2023. blaNDM gene presence was confirmed by PCR. MIC values were determined for aztreonam, cefiderocol, ceftazidime-avibactam (CZA), colistin, ertapenem, imipenem and meropenem. All strains were typed by XbaI-PFGE and 8 samples (one for each pulsotype) were sequenced with a hybrid method by Oxford Nanopore MinION and Illumina MiSeq technology. Reads assembly was performed by Unicycler v 0.4.8.

Results: Resistance to antibiotics was 100% for CZA, ertapenem and meropenem, 94% for imipenem, 88% for aztreonam, 35% for cefiderocol and 12% for colistin. PFGE showed 8 different restriction profiles and the more represented one (7 samples) occurred from May 2022 to August 2023. WGS revealed 5 sequence types (STs): STs 11, 147 and 395 consists of 2 samples each while the remaining 2 isolates belonged to STs 307 and 437. blaNDM-1 allelic variant characterized STs 11, 147 and 307 while blaNDM-5 the other two. blaNDM was localized on plasmids with different sizes (from 79 to 370 kb) and Inc groups (IncFIB, IncHI1B, IncFII, IncFIA) except for one ST395 in which was chromosomally encoded.

Discussion and conclusions: This study presents the first detection of Kp-NDM in our regional hospital. Samples were collected from different sources but 8 out of 17 were rectal swabs, underlining the ability of these clones spreading out of healthcare settings. Moreover, the variety of plasmids carrying blaNDM highlighted the high mobility of this gene as well as its detection in different clones,

some of which caused outbreaks in other hospitals. In Tuscany, NDM-Kp ST147 was linked to outbreaks since 2018 and in 2022 increasing numbers were registered in Germany, pointing out the global spread of this clone. Two plasmids belonging to samples of STs 147 and 307 share 99,9% of identity and 90% of coverage with a 367 kb element of Kp ST383 carrying blaNDM-5. This clone coharbour blaNDM-5 and blaOXA-48 in distinct plasmids and was responsible of an outbreak in our hospital from September 2022, suggesting a possible spread of blaNDM through different Kp strains.

223 - IN VITRO MODELS TO ASSESS AUTO-ABS NEUTRALIZING THE PROTECTIVE EFFECT OF TYPE I IFNS AGAINST WNV INFECTION

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In vitro models to assess auto-Abs neutralizing the protective effect of type I IFNs against WNV infection

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Introduction

Mosquito-borne West Nile virus (WNV) infection is asymptomatic or causes a mild febrile illness in the great majority of individuals, but can cause WNV neuroinvasive disease in ~1:150 infected individuals.

Auto-antibodies (auto-Abs) neutralizing type I IFNs underlie ~15% of hypoxemic COVID-19 pneumonia, ~5% of critical influenza pneumonia, ~25% of MERS pneumonia and ~30% of adverse reactions to the Yellow Fever D17 vaccine strain. We recently identified auto-Abs in ~40% of individuals with WNV neuroinvasive disease. In vitro models to assess auto-Abs neutralizing the protective effect of type I IFNs against WNV infection are crucial to define their pathogenic role and screen patients.

Material and methods

Circulating IgG-auto-Abs against type I-IFNs had been previously detected by ELISA in 102 sera from WNV infected subjects enrolled in Pavia. In vitro WNV infection in human retinal pigment epithelial cells (ARPE-19) was assessed using different IFN-α2 concentrations with serial dilutions of sera from patients with WNV disease carrying auto-Abs against IFN-α2 (n=5) and IFN-ω (n=4). Sera from patients with WNV disease without anti-type I IFN auto-Abs were used as controls. The experiments were carried-out in parallel on Vero E6 cells

Results

We show that IFN-α2 and IFN-ω protect ARPE-19 cells from WNV infection in vitro. ARPE-19 cells

show similar sensitivity to IFN-α2 compared to Vero E6 cells. Serum samples from patients with auto-Abs block the antiviral function of IFN-α2 and/or and IFN-ω in ARPE-19. In experiments to test serial dilutions, the sera maintained their blocking effect up to a 1:300 dilution, while no effect was observed using sera without auto-Abs.

Discussion and conclusion

Our preliminary data show that the human ARPE-19 cell line is as reliable cell line to test sera from patients with WNV disease for neutralizing anti-type I IFN auto-Abs. Our findings also confirm that neutralization of type I IFN antiviral immunity is a major mechanism of life-threatening viral disease. The ARPE-19/WNV cell model should be validated for testing of samples from patients with infection other than WNV, including COVID-19, influenza pneumonia and MERS. Our experiments are also a starting point to test other human cells for sensitivity to type I IFNs and the blocking effect of sera from patients with life-threatening viral disease.

261 - SYNERGISTIC EFFECT OF P-BROMODOMIPHEN BROMIDE WITH CONVENTIONAL ANTIFUNGALS AGAINST C. AURIS.

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Synergistic effect of p-bromodomiphen bromide with conventional antifungals against C. auris.

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Background - Candida auris is an emerging opportunistic yeast which can develops biofilms allowing its persistence on environmental surfaces and human niches. Most C. auris strains (90% of isolates) display fluconazole (FLZ) resistance, 30% resistance to amphotericin B (AmB) and 5% to echinocandins. The limited therapeutic options have prompted researchers to explore drug combinations or potential alternatives for C. auris treatment. Quaternary ammonium compounds (QAC) are known for their biocidal activity. Domiphen bromide (D-Br) has been shown to potentiate triazole activity against C. albicans. Its derivative, p-bromodomiphen bromide (B-Br), has been recently enlisted as a new, potent, and fast-acting QAC. In this study, we tested B-Br and D-Br against C. auris, in both planktonic and sessile cells, in combination with FLZ or AmB.

Methods – Two clinical strains of C. auris with different antimicrobial susceptibility patterns (CaS, resistant to FLZ; CaR, resistant to FLZ and AmB) were used. FLZ (512.0-0.25 µg/mL), AmB (8.0-0.03 µg/mL), and B-Br (64-0.03 µg/mL) were used alone or in combination. Broth microdilution and XTT assays were used for planktonic and sessile antimicrobial susceptibility testing, respectively. Checkerboard tests were performed to study the interaction between QAC and conventional antifungals. To assess the cytotoxicity of B-Br, MTT assay was performed on HaCat and Hep-2 cell lines.

Results – Fluconazole planktonic MICs were >256.0 µg/mL and 128.0 µg/mL, respectively for CaR and CaS, whereas for AmB MICs were 2.0 and 1.0 µg/mL. For B-Br, MICs were 4 µg/mL and 2 µg/mL for CaR and CaS, respectively. When testing C. auris biofilms, fluconazole MICs progressively increased from adhesion to mature biofilm up to >512.0 µg/mL, whereas for AmB MIC were stable. The fractional inhibitory concentration index (FICI) indicated a synergistic effect of B-Br with both FLZ and AmB against CaS and CaR biofilms (FICIAmB/B-Br=0.37 and FICIFLZ/B-Br =0.5 for both sessile isolates).

Conclusions – B-Br synergize with FLZ and AmB resulting in a stronger inhibitory activity also against C. auris biofilms, without exerting toxicity. In combination, the concentration of the FLZ and AmB fell within the range of concentrations considered effective in clinical practice.

262 - THE ROLE OF RSV VIRAL LOAD AND CO-INFECTION ON DISEASE SEVERITY IN A VERY LARGE COHORT OF PAEDIATRIC PATIENTS

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The role of RSV viral load and co-infection on disease severity in a very large cohort of paediatric patients

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Background

Respiratory syncytial virus (RSV) is a common cause of acute respiratory infection (ARI) of the lower respiratory tract in young children, with a particularly high burden of disease in individuals <2years of age. Despite this, the clinical impact of co-diagnosis of RSV with other respiratory viruses is not well understood. We aimed to explore the frequency of RSV in mono and co-infection and clinical outcomes associated with RSV infection in paediatric population.

Material and Methods

A retrospective analysis of paediatric patients admitted at Bambino Gesù Children's Hospital in Rome who had a RSV positive respiratory sample screened by The Alleplex Respiratory Panel assay (Seegene) between January 1, 2022, and December 31, 2023 was performed.

Results

Among 13,952 samples collected during the study period, 492 (3.5%) were RSV-positive. Of these, 466 belonged to paediatric patients (Median [IQR] age: 0.4 [0.1-2.1] years) with ARI diagnosis at hospital

admission. Four hundred and five (86.9%) involved the lower-respiratory tract, while only 61 (13.1%) involved the upper-respiratory tract. Viral co-infections were mainly observed in the upper-respiratory tract infections (71.7%) with respect to the lower (68.6%), (P=0.071). However, considering clinical manifestation, in the lower-respiratory tract the RSV co-infection was mainly observed in patients with pneumonia (21.0%) while RSV mono-infection mainly characterized bronchiolitis (89.4%) (P=0.021). Notably, RSV cycle-threshold (CT) value was significantly lower in mono-infection (median [IQR]: 21.3 [17.7-25.0]) than in co-infection (23.1 [19.1-29.0]) (P=0.003). Logistic regression showed that the highest CT value was positively associated with RSV co-infection (adjusted odds ratio, AOR [95%CI]: 1.07 [1.00-1.13], P=0.008), while a negative association was observed with bronchiolitis (AOR [95%CI]: 0.32 [0.17-0.57], P<0.001).

Conclusions

These results showed that RSV mono-infection with high viral load mainly characterized bronchiolitis. Conversely, a lower viral load and pneumonia were observed when RSV was in co-infection, thus suggesting that the presence of another virus can potentially reduce the replicative capacity (and perhaps pathogenicity) of this virus. RSV co-infection is associated with low viral load, and a specific clinical outcome. Therefore, the quantitative study of RSV is important in combination with virus-virus interactions dynamics.

265 - SET-UP OF A COMPREHENSIVE 3D CELLULAR MODEL TO STUDY SARS-COV-2 GI TRACT INFECTION.

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Set-up of a comprehensive 3D cellular model to study SARS-CoV-2 GI tract infection.

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Introduction: SARS-CoV-2 is the etiological agent of COVID-19 which is mainly considered a respiratory disease. Notwithstanding, viral infection can be associated to systemic pathologies affecting major organs including gastrointestinal (GI) tracts. Several studies indicate that GI symptoms are common in COVID-19 patients, with persistent viral activity in the gut even after its clearance from the respiratory tract. Recently, organoids (OGs) are used as cellular model to faithfully reproduce the condition of the organ of derivation and mimic its response to various stimuli. The aims of this study are to establish an efficient SARS-CoV-2 infection in organoid cultures representatives of different GI tracts and to compare the different infection rate and related cellular modifications.

Materials and Methods: We set up extraction and expansion protocols of organoids derived from stomach, duodenum, colon, and colon-rectum human biopsies. Organoids were dissociated to single cells using TryPLE Express Enzyme (Thermo Fisher Scientific), and SARS-CoV-2 infected at MOI 10. After 2 hours of virus adsorption, cells were washed to remove unbound virus. To restore the 3D-culture, cells were re-embedded into 40µL Cultrex™ Ultimatrix BME (Bio-Techne) and cultured in 500µL expansion medium. At 48 and 72 hours post infection (hpi) supernatant and cells were harvested. We determined supernatant and intracellular viral load through Real-Time PCR to evaluate cells capability to support SARS-CoV-2 viral infection and replication as well as Angiotensin Converting Enzyme 2 (ACE2) receptor expression, to understand different GI tracts susceptibility to the virus.

Results: Our preliminary results demonstrate an efficient SARS-CoV-2 infection in all GI tracts analyzed. We observed a higher ACE2 basal gene expression in duodenum compared to colon, gastric and rectal OGs. At 48hpi, ACE2 gene expression slightly decreases in all the GI tracts analyzed, except for rectal OGs. Moreover, duodenum OGs showed a higher SARS-CoV-2 ORF1ab gene expression at 72hpi when compared to the other GI tracts analyzed. In addition, we evidenced a correlation between ORF1ab gene expression at 72hpi and basal ACE2 gene expression.

Discussion and Conclusions: Overall, the efficient infection reported in all the OGs analyzed suggest that GI might have an active role in SARS-CoV-2 manifestations, with a prominent infection in duodenum, in accordance with clinical implications previously reported. Our data support the use of GI organoids as a model for studying the implications of SARS-CoV-2 in gastrointestinal symptoms. Furthermore, the protocol we have established could be expanded to the study of the impact of other viruses on the gastrointestinal tract based on 3D cellular model.

269 - PREVALENCE OF NTM INFECTIONS IN A REFERRAL LABORATORY IN ITALY AND CORRELATION BETWEEN CULTURE AND MOLECULAR RESULTS AND CLINICAL CONDITIONS.

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Prevalence of NTM infections in a referral laboratory in Italy and correlation between culture and molecular results and clinical conditions.

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Background: Diagnosis of NTM disease can be challenging for several reasons, including the ubiquitous nature of these species. Interpretation of the results obtained with the classical microbiological diagnosis tools (culture and molecular tests) shall ideally help to distinguish a potential disease from a possible contamination of the biological sample. In this study, we provide the data obtained in our referral center, where we perform diagnosis of NTM by using a combination of culture and molecular assays.

Methods: We retrospectively analyzed data on the isolation of Mycobacterium species from biological samples received over the last 5 years (2018 to 2023) in our referral center at the Laboratory of Microbiology of the Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome. Standard culture and molecular tools were used to detect and identify NTM species. The Anyplex MTB/NTM MDR-TB was used to differentiate NTMs from the Mtb complex; a PCR-based sequencing technique was used for the identification of mycobacterial species by sequencing the hsp65 gene.

Results: We observed a steady increase in the number NTM strains isolated in culture during the time considered. Since 2018 to 2022 we reported a 7 fold-increase. Species identification indicated a prevalence of NTM of the MAC group, that accounts for approximately 65-85% of the total NTM depending on the year analyzed. In 2022 and 2023, we observed a significant increase in the number of M. abscessus strains isolated (from 0 % in 2020 to 5.81 % in 2022). Determination of bacterial loads in the clinical samples may be of clinical significance, given the potential presence of NTM as "contaminants". We correlated the Ct obtained with a commercial RT-PCR for mycobacteria with the

time to positivity (TTP) obtained in culture (MGIT) and these data with the clinical conditions observed (asymptomatic, established disease, previous illness).

Conclusion: This study indicates a growing trend in the incidence and prevalence of NTM. We highlight an increase in relapses in the last 2 years linked to the lack of adequate therapies (from 2.6 % in 2021 to 21% in 2023) and the clinical relevance of RT-PCR in the diagnosis of NTM disease.

275 - AN OVERVIEW OF ITALIAN MICROBIAL CULTURE COLLECTIONS: THE SUS-MIRRI.IT PROJECT.

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An overview of Italian Microbial Culture Collections: the SUS-MIRRI.IT project.

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1Introduction. Culture Collections (CCs) and Microbial Biological Resource Centres (mBRCs) are repositories of microorganisms that study and conserve biodiversity and facilitate the access of the scientific and industrial communities to microbial strains by providing skills and services to external users.The main objectives are the collection, characterisation, preservation and distribution of microbial strains, both for research and bioindustry purposes, together with the exchange of related metadata. The aim of this work is to provide an overview of the current status of Italian CC and mBRCs, highlighting their strengths, weaknesses and future opportunities.

2Materials and Methods.The data presented were collected and compared through two surveys prepared and distributed to the Italian CCs within the JRU(Joint Unit Research) MIRRI-IT (Microbial Resource Research Infrastructure-Italy) and SUS-MIRRI.IT project.

3Results.The Italian CCs are 31 and belong to both universities and public research organizations. They are part of national or international networks, that promote collaboration and the exchange of both management and scientific ideas and information. Networking enables the creation of synergies in tackling common challenges. The JRU MIRRI-IT was set up between the Universities of Turin, Perugia, Modena and Reggio Emilia,the AOU San Martino in Genoa and the CNR.JRU coordinates the European project “Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy” (SUS-MIRRI.IT).The activities of MIRRI-IT include:microbial identification, characterisation and application; microbiomes;common procedures and protocols;collection data management;biosecurity;national plan on biodiversity of agri-food interest;promotion and communication.The collected microorganisms consist of 102,405 strains (7,000 species) in public (74%), secure (34%) or patent (10%) repositories.Their identification and characterisation is carried out using molecular techniques (43%),NGS (Next-Generation Sequencing,24%), genotyping (48%):the use of MALDI-TOF mass spectrometry (now at 25%) will be implemented in the coming years.CCs also constitute research infrastructures that provide:services (68% of which 90% fee-paying) and expertise

to research institutes and companies; training courses in microbiology.SUS-MIRRI.IT is working on the development of an open source interoperable national catalogue of microbial resources (ItCCC) for the management and administration of all datasets.

4Discussion and Conclusions. CCs and mBRCs can facilitate scientific research, but they can also be extremely helpful in achieving the United Nations Sustainable Development Goals: access to microbial resources plays a crucial role in several sectors of the bioeconomy.

281 - BORDETELLA PERTUSSIS IN THE EMILIA ROMAGNA REGION: MICROBIOLOGICAL DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF THE CIRCULATING STRAINS

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Bordetella Pertussis in the Emilia Romagna Region: microbiological diagnosis and molecular characterization of the circulating strains

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Introduction. The study describes the circulation of Bordetella pertussis (Bp) strains in the Emilia Romagna Region (ERR) highlighting the role of microbiological diagnosis and molecular characterization of the strains involved.

Material and Methods. From December 2013 to date, a total of 644 pertussis suspected cases was reported in ERR (median age: 13 years; range: 1 day-87 years); 785 samples (141 sera and 644 nasopharyngeal swabs/aspirates, NPS/NPA) from these patients were analyzed. Serological analysis (IgG[IgA] anti-Bp detection) was performed by the LIAISON® Bordetella pertussis Toxin IgG/IgA kit (DiaSorin). Bp-DNA detection was carried out using the Simplexa Bordetella Direct kit (DiaSorin), targeting the IS481 region common to both Bp and Bordetella holmensii. Positive results were confirmed by the Bordetella ELITe MGB® Kit, identifying a specific Bp DNA region (ptxA). Culture method was performed in 448/644 cases. Finally, molecular characterization of Bp strains was performed by Sanger sequencing, analyzing the promoter region of the pertussis toxin gene (ptxP), the coding sequence for toxin S1 subunit (ptxA) and pertactin gene (prn).

Results. A total of 126/644 (19.5%) cases was confirmed by a positive result in at least one of the detection methods. Among these, serological test was carried out on 55/126 (43.6%) cases, obtaining a positive result in 33 (60%) cases. Bp-DNA was detected in 116/126 (92%). Culture method, performed in 69/126 (54.7%) cases, resulted positive for 24.6% (17/69). The molecular tests were positive in all NPS/NPA samples collected within 4 weeks of symptom onset (43/126, 34.1%); of these, 36 samples underwent culture method, obtaining Bp isolates in 22.2%. For 6 cases, Bp infection was confirmed exclusively through serological testing: all showed symptoms for more than 1 month after sample collection. The molecular characterization of Bp strains was performed for the 41.3% of cases resulted positive by molecular method, showing the circulation of strains with ptxP3-ptxA1-prn2 genetic profile, combined in 62.8% of cases with mutations associated to pertactin deficient gene.

Clinical data were available for 71.5% of cases, demonstrating a severe clinical course in 20.5% of cases; exitus occurred in 2 patients under 1 month of age.

Discussion and conclusions. The obtained results showed: i) Bp circulation in the ERR remains a cause of infant mortality, ii) circulation of Bp strains with pertactin deficient gene mutations (a component of anti-Bp vaccines); iii) the time elapsed between symptom onset and sample collection is crucial for an accurate microbiological diagnosis.

T06 RISPOSTA DELL'OSPITE ALLE INFEZIONI E ALLE VACCINAZIONI

2 - EPSTEIN-BARR VIRUS EBNA1 EPITOPES INDUCE EAE-LIKE SYMPTOMS IN PARK2 KNOCKOUT MICE: IMPLICATIONS FOR MITOCHONDRIAL DYSFUNCTION AND NEUROINFLAMMATION

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Epstein-Barr Virus EBNA1 Epitopes Induce EAE-like Symptoms in PARK2 Knockout Mice: Implications for Mitochondrial Dysfunction and Neuroinflammation

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Abstract: This study investigates the intricate interplay between mitochondrial dysfunction, infections, and neuroinflammation, with a particular focus on the impact of pathogenic epitopes of Epstein-Barr Virus (EBV) nuclear antigen 1 (EBNA1) in PARK2 knockout mice, which exhibit mitochondrial deficiencies. The investigation involved mice subjected to experimental autoimmune encephalomyelitis (EAE) induction using myelin oligodendrocyte glycoprotein (MOG)35-55 peptide. Following immunization with EBNA1, only PARK2-/- exhibited symptoms resembling EAE, the severity of which was comparable to MOG immunization. Notably, PARK2-/- mice exhibiting EAE-like symptoms after EBNA1 immunization showed an increased percentage of monocytes and dendritic cells in the spleen, and an increased astrocytes and microglia activation in the brain and spinal cord compared to placebo controls or to mice immunized with MOG35-55. Furthermore, following EBNA1 administration, both knockout and wild-type mice displayed significant macrophage and plasma cell infiltration in the intestine, along with increased mitotic activity of crypt epithelial cells and dysplasia of epithelial cells. These findings highlight the role of EBV in exacerbating inflammation, particularly in the context of mitochondrial deficiencies and aging.

14 - KIR2DL2 AND HERPESVIRUS INFECTION AMONG INDIVIDUALS WITH MULTIPLE SCLEROSIS: IMPLICATIONS FOR THE DYNAMICS OF THE T CELL RECEPTOR REPERTOIRE.

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KIR2DL2 and Herpesvirus infection among individuals with Multiple Sclerosis: implications for the dynamics of the T cell receptor repertoire.

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Introduction: For a considerable time, infection has been considered a possible initiator or worsening factor in the onset and advancement of multiple sclerosis (MS). Although the exact mechanisms are not fully understood, numerous infectious agents, including viruses like Herpes simplex 1 (HSV-1), Epstein-Barr virus (EBV), and human herpesvirus 6 (HHV-6), have been studied regarding their potential link to MS. Research indicates a connection between specific KIR genes, like KIR2DL2, and the risk of developing MS, where differences in KIR gene patterns might influence NK cell activity and the equilibrium of immune responses. More specifically, KIR2DL2 has been linked to changes in NK cell cytotoxicity and the production of cytokines, potentially contributing to the immune dysregulation and increased infection susceptibility seen in MS.

Aim: This research delves into the intricate relationship between viral infections, immune cell responses, and genetic factors in the context of MS.

Methods: We investigated the impact of Herpesvirus (HHV) infection on MS patients (specifically those with Relapsing-Remitting MS), assessing IgG anti-HHV levels, HHV viral load, the effects of HHV infection on microglial cell activation, and the T cell receptor repertoire.

Results: The results obtained confirm a link between viral infections and the expression of the KIR2DL2 gene in MS patients, particularly involving herpesviruses such as Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6). Individuals with MS and other inflammatory neurological disorders (OIND) show increased prevalence of KIR2DL2 gene expression, correlating with higher viral load and altered viral subtype distribution. Moreover, there is an increase in late-differentiated T cell subsets and varied

activation states of T cells in KIR2DL2 positive MS patients, suggesting a complex relationship involving viral infections, immune cell exhaustion, and disease progression.

Conclusions: Analysis of the T-cell receptor (TCR) repertoire reveals intriguing patterns in T-cell diversity and clonality associated with KIR2DL2 expression, indicating a more personalized and diverse T-cell response profile in KIR2DL2 positive patients. These findings highlight the potential role of KIR2DL2 in shaping innate and adaptive immune responses and underscore the importance of antigen-specific T-cell responses in MS pathogenesis.

22 - BRAKE AND THROTTLE IN ONE PEDAL: PREVENTING SEPTIC SHOCK WHILE AUGMENTING HOST DEFENSES AGAINST BACTERIAL INFECTIONS BY CASPASE-8 INHIBITION

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Brake and throttle in one pedal: preventing septic shock

while augmenting host defenses against bacterial infections by caspase-8 inhibition

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Introduction: There is a pressing need to develop host-directed therapies to treat invasive infections by antibiotic-resistant bacteria. However, traditional approaches at augmenting host defenses have been hampered by the risk of inducing cytokine storms and septic shock. We show here that caspase-8 is a crucial mediator of septic shock and that pharmacological caspase-8 inhibition ameliorates this condition. At the same time caspase inhibition unleashes a neutrophil-centered response leading to bacterial clearance.

Materials and Methods: The therapeutic activities of the caspase-8 inhibitor z-IETD-fmk were assessed in mouse models of bacterial pneumonia and septic shock. Host responses were analyzed by measuring neutrophil numbers and cytokine concentrations in body fluids and organ homogenates.

Results: Pretreatment with z-IETD-fmk prevented hypothermia and death in septic shock models in which mice were inoculated with a lethal dose of LPS or live Streptococcus agalactiae.Treatment with the caspase inhibitor also ameliorated the outcome of Streptococcus pneumoniae or Klebsiella pneumoniae pneumonitis by augmenting the number of circulating neutrophils, neutrophil influx to the lung and bacterial clearance. Neutrophilia and neutrophil recruitment were due to interleukin-1 beta (IL-1β)-driven release of C-X-C motif chemokine receptor 2 (CXCR2) agonists and granulocyte colony stimulating factor (G-CSF). Moreover, z-IETD-fmk was sufficient to drive, by itself, the production of several chemokines, cytokines and growth factors by a signal transduction pathway involving tonic tumour necrosis factor-alpha (TNF-α) production in neutrophils and receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and RIPK3 activation.

Discussion and Conclusions: We show that acute inhibition of caspase-8 unleashes a constitutive proinflammatory pathwayheld in check by this protease, leading to chemokine release and neutrophil mobilization to infection sites. At the same time, caspase-8 inhibition prevents uncontrolled apoptosis which is a central feature of septic shock. Therefore caspase-8 inhibition is a promising host-directed approach to treat severe bacterial infections.

23 - CONTRIBUTION OF FORMYLATED PEPTIDE RECEPTORS TO IMMUNE SENSING OF GROUP B STREPTOCOCCI BY HUMAN NEUTROPHILS

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Contribution of formylated peptide receptors to immune sensing of Group B streptococci

by human neutrophils

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Introduction: Neutrophil recruitment to infection sites is of central importance in the control of Group B streptococcal and other bacterial infections. However, the bacterial stimuli capable of activating neutrophil-mediated pathogen sensing and effector responses against infection are incompletely characterized. In the present study we evaluated the ability of a set of formylated peptides produced by Group B streptococcus (GBS) to activate neutrophils and analyzed the underlying mechanisms.

Materials and Methods: We examined the ability of GBS N-formyl peptides to activate formyl peptide receptors (FPR1 and FPR2) in human neutrophils isolated from the peripheral blood of healthy donors by looking at gene induction and neutrophil chemotaxis.

Results: High-level production of interleukin-8 (IL-8) in neutrophils was elicited only by live, but not dead bacteria, and required both FPR1 and FPR2, as demonstrated using specific inhibitors. Moreover, we identified two novel group B streptococcal formylated peptides, each activating respectively FPR1 and FPR2, namely fPep8 and fPep10, which were capable to potently activate human neutrophils. These peptides promoted strong chemotactic responses and synergize with bacterial toll-like receptor (TLR) agonists in induction of high levels of IL-8. In addition, we found that FPR sensing underlies the ability of neutrophil to discriminate between live and dead bacteria.

Discussion and Conclusions: We identified two novel formylated peptide sequences in GBS which induce neutrophil chemotaxis and synergize with TLR agonists. Collectively, our data highlight the presence of a highly integrated detection system in neutrophils, whereby FPR1, FPR2 and TLRs cooperate in sensing the presence of viable GBS. The augmentation of the functional activities of these receptors may represent a new alternative therapeutic strategies to treat antibiotic-resistant bacterial infections.

26 - A THREE-YEAR INTERVENTIONAL STUDY ON DIFFICULT-TO-TREAT PSORIATIC PATIENTS TREATED WITH APREMILAST AND ITS ROLE IN FUNGAL COLONISATION

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A three-year interventional study on difficult-to-treat psoriatic patients treated with apremilast and its role in fungal colonisation

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Introduction: Fungi, as Candida and Malassezia, have been shown to be responsible for the exacerbation of cutaneous and arthritic psoriasis through the catelicidin pathway, which leads to increased production of interleukin (IL)-23, which in turn activates IL-17 pathway. Especially, in difficult-to-treat psoriasis, fungi could act as an inflammatory trigger perpetuating IL-17/23 axis.

Materials and Method: We enrolled 70 patients in three years, followed for 52 weeks with follow up visits every 12 weeks, to evaluate the prevalence of Candida species and other opportunistic fungi in psoriatic patients with “difficult-to-treat areas” and the impact of apremilast, a phosphodiesterase inhibitor, on i) Candida colonisation rates after treatment with swabs and next generation sequencing methods ii) its clinical efficacy on psoriasis iii) its effect on serum interleukin (IL)-17, -10, TGF-β and biochemical serum profile.

Results: Among 70 enrolled patients, mucosal and cutaneous samples collected at T0 from 70 psoriatic patients were positive for the presence of fungal pathogens. Candida was detected in the oral cavity of 24 (34%) patients and in stool samples of 9 (13%) patients. The most common species was C. albicans, isolated in all 33 patients. Interestingly, of 33 colonized patients, 13 patients had a combination of two or more Candida spp. in the oral cavity. We observed a high prevalence of Candida ssp. in patients with comorbidities (Chi-Square; $p<0.05$), in particular with neoplasms in anamnesis, cardiovascular comorbidities and a high BMI (> 25 Kg/cm2). Interestingly, apremilast at week 52 was able to eliminate the fungus in the majority of patients (83%) who were colonized by only C. albicans; whereas in those co-infected by two or more Candida spp. the drug was not able to eradicate the fungus despite a significant reduction (t-test; $p<0.05$). Furthermore, Malassezia spp. was isolated from skin lesions in 32/38 patients (85%) at baseline, and in 7/38 (18%) after 52 weeks of treatment. The data suggest a significant high rate of oral colonization by Candida albicans and skin colonization by Malassezia in psoriatic patients at baseline condition. Moreover, just after 16 weeks, all the patients presented clinical improvements, directly related to the reduction of Candida spp. and Malassezia

spp. colonization. Furthermore, IL-17, IL-10 and TGF- β presented a reduction after 52 weeks of treatment.

Discussion and Conclusions: Candida may play a possible role as a trigger factor in the pathogenic process of this disease. By dampening inflammation, apremilast may arrest the “vicious cycle” triggered by Candida itself, thus restoring gut homeostasis in psoriatic patients. Apremilast treatment resulted in the reduction of Candida spp. and Malassezia spp. prevalence without the use of antifungals; in clinical improvement of PASI, NAPSI, DLQI, itching, and in modulation of serum pro- and anti-inflammatory cytokines.

40 - NEUTRALIZING ANTIBODY RESPONSE TO THE HIGHLY DIVERGENT BA.2.86 SARS-COV-2 LINEAGES IN VACCINATED HEALTH CARE WORKERS WITH OR WITHOUT SUBSEQUENT INFECTION.

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Neutralizing antibody response to the highly divergent BA.2.86 SARS-CoV-2 lineages in vaccinated Health Care Workers with or without subsequent infection.

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Introduction. Since the emergence of the Omicron variant, SARS-CoV-2 has been evolving into a constellation of related lineages. One key issue is whether past natural and/or vaccinal immunity remains effective against latest lineages. Aim of this work was to investigate in a live virus in vitro assay, the neutralizing antibody (NtAb) response against BA.2.86 and ancestral B.1 SARS-CoV-2 in a cohort of previously vaccinated health care workers (HCWs) with or without following infection, enrolled in the EuCARE consortium.

Materials and Methods. Two-fold serial dilutions of heat-inactivated sera were incubated with 100 TCID50 of each SARS-CoV-2 virus stock at 37°C for 1 h and added to 10,000 pre-seeded Vero E6 cells per well in 96-well plates. After 72 h at 37°C, cell viability was determined through the CellTiter-Glo® Cell Viability Assay (Promega). The NtAb titer was defined as the reciprocal value of the sample dilution showing a 50% protection of virus-induced cytopathic effect. Each run included a mock control, virus back titration and a known SARS-CoV-2 neutralizing serum. Statistical analyses were performed using IBM SPSS software.

Results. A total of 68 HCWs (49±11 years, male 12%) were selected, including 47 (51±10 years) SARS-CoV-2 vaccinated with 4-5 doses but never infected and 21 (44±10 years) vaccinated with 3-5 doses and then infected during the early Omicron waves. Globally, 77% HCWs received mRNA vaccines and 24% received the bivalent (BiV) vaccination. Sera were collected at median 119[17-137] days since the last immunization event, with a significant difference between groups (167[18-229] vs. 114[16-129] days in infected vs. uninfected, respectively; p=0.012). Overall, NtAb titres to B.1 were significantly higher than to BA.2.86 (1201[572-2518] vs.164[56-437]; p<0.001). Four individuals lacked measurable NtAbs against BA.2.86. The uninfected group had significantly higher NtAb titres compared with the infected group, both against B.1 (1440 [906-4415] vs. 349[271-1162] p<0.001) and against BA.2.86 (231[96-470] vs. 93[40-176] p=0.003). Overall, no significant correlation between days since last immunization and NtAb levels to B.1 (rho = -0.096; p =0.437) and BA.2.86 (rho=-0.169; p=0.169) were observed. NtAbs titers were comparable between HCWs receiving monovalent vs. BiV vaccination against B.1 (1179[591-2740] vs.1228[404-2422]; p=0.795) and BA.2.86 (135[47-320] vs. 174[56-486]; p=0,301).

Discussion and Conclusion. Almost 1-log reduction in NtAbs titres to the highly divergent BA.2.86 virus vs. the ancestral B.1 was observed in HCWs vaccinated or vaccinated and infected. Ongoing analyses on a larger number of samples associated with studies of cell-mediated immunity will enrich the data on NtAb response clarifying the breadth and duration of immunity to SARS-CoV-2 variants.

71 - TRANSCRIPTIONAL ACTIVITY OF HERV FAMILIES AND INTERLEUKIN GENES UNDER SIMULATED MICROGRAVITY

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Transcriptional Activity of HERV Families and Interleukin Genes Under Simulated Microgravity

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Introduction: Endogenous retroviruses make up about 8% of the human genome (ERVs). Most of them are normally maintained in a silenced state, but proviral RNA or protein has been detected in various disease contexts such as cancer, autoimmune, and inflammatory disorders. Interestingly, it has been reported that stress conditions facilitate ERV expression. Space travel will expose astronauts to microgravity environments (a stress condition), which may result in activation of ERVs and might influence pathogenic outcomes during space flight. This study aimed to elucidate the transcriptional activity of three HERV families (W, K, H) and interleukin genes (IL-1, IL-6, and TNF) in different cell lines under microgravity (MG) conditions and compare them with the results obtained in the same cells under normal gravity (NG; 1G). Materials and methods: Different cell lines (neuroblastoma cells: M17 and SH-SY5Y, cancer cells: HEp-2 and Caco-2 cells), were seeded (1×10^5 cells) in eighteen T12.5 flasks and incubated at 37°C in a humidified incubator with 5% CO2 until they reached 90% confluence. Eight flasks were incubated under MG conditions (random positioning machine RPM, a device simulating MG), and eight flasks under NG conditions. The cells were harvested at different times: 0 (n: 2 flasks), 3 h (n: 2 flasks), 6 h (n: 2 flasks), 24 h (n: 2 flasks), and 48 h (n: 2 flasks), and total RNA was extracted. The cDNA was synthesized using a Reverse Transcription kit with random hexamer primers. ERVs (HERV-K, HERV-W, and HERV-H) and interleukin genes (IL-1, IL-6, and TNF) expression was investigated by real-time PCR. Statistical analysis of the data was done using GraphPad Prism version 8 software, and fold change was calculated. Results: In all cell lines, the expression of IL-1, IL-6, and TNF was increased after 3 hours in MG and remained elevated after 6, 24, and 48 hours in MG. In parallel, HERV-W and HERV-K were significantly upregulated after 3 and 6 hours in neuroblastoma cells exposed to the MG, whereas after 24 hours, the expression decreased in SH-SY5Y. The HERV-H gene expression did not change when neuroblastoma cells were exposed to MG. Moreover, in HEp-2 cells, the expression of HERV-H, K, and W genes was significantly increased when exposed to short-term MG (3 h, 6 h) and remained elevated at 24 h and 48 h of MG exposure. In Caco-2 cells, HERV-K gene expression was significantly higher after 3 h, 6 h, and 24 h of RPM exposure compared to NG. Taken together, the dysregulation of interleukins and HERV genes was observed under stimulated MG, which demands further investigation for human health protection in space.

87 - STRAIN-LEVEL VARIATION WITHIN HOST-SPECIFIC MICROBIOTA MODULATES HUMORAL RESPONSES TO VACCINATION IN MICE

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Strain-level variation within host-specific microbiota modulates humoral responses to vaccination in mice

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Introduction: Vaccine-induced immune responses exhibit significant variability, which has been also attributed to the impact of the gut microbiota. While broad associations between microbiota and immunity are recognized, the influence of specific bacterial taxa on IgG antibody responses to vaccination remains less well-defined. We previously showed that in outbred mice the IgG response to subunit vaccination depends on the gut microbiota composition, influencing immune activation and the expression of co-signaling molecules. This work aims to further investigate the impact of selected taxa and the importance of species-specific host-microbiota interactions in shaping the humoral response to vaccination.

Materials and Methods: We vaccinated 6-week-old Swiss Webster (SW) (outbred) and C57BL/6 (inbred) specific pathogen–free (SPF), antibiotic-treated, and germ-free (GF) mice with a glycoconjugate antigen model two times at two weeks interval using alum as adjuvant. Antigen-specific antibody responses were monitored in sera using ELISA, while cellular immune responses were assessed in lymph nodes and spleens via flow cytometry. Bioinformatic analysis, including Analysis of Composition of Microbiomes (ANCOM), was used to identify taxa linked to heightened IgG responses following vaccination. GF mice were colonized with either single or complex bacterial colonies via oral gavage.

Results: Bioinformatic analyses revealed a strong association between Enterococcus faecalis and Prevotella species with IgG vaccine-responder groups, which was further validated by colonization experiments. Notably, distinct strain-level differences were observed in the ability of selected taxa to modulate immune responses. Colonization experiments with SW and C57BL/6 mice demonstrated that variations in immune response modulation are closely tied to the genetic background of the host and the specific microbial strain introduced.

Discussion and Conclusions: Our findings underscore the significance of specific microbial taxa on vaccine-induced IgG responses and highlight the critical role of host-microbiota specificity. This study enhances our understanding of the microbiota's impact on immune modulation and opens promising avenues for microbiota-targeted interventions.

113 - SERUM FROM COVID-19 PATIENTS PROMOTES ENDOTHELIAL CELL DYSFUNCTION THROUGH PROTEASE-ACTIVATED RECEPTOR 2

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Topic: Risposta dell'ospite alle infezioni e alle vaccinazioni

Serum from COVID-19 patients promotes endothelial cell dysfunction through protease-activated receptor 2

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Introduction: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection generates an inflammatory response able to alter the capacity of the endothelium to regulate vascular tone, immune responses, and the balance between anti-thrombotic and pro-thrombotic properties. Although it is widely recognized that endothelial dysfunction plays a central role in the pathophysiology of Coronavirus Disease (COVID)-19 and is closely linked to the severity and mortality of the disease, the specific endothelial pathways altered during COVID-19 still need to be fully clarified. Thus, the aim of this study was to identify the molecular mechanisms underlying the endothelial dysfunction following SARS-CoV-2 infection. To this purpose, we compared the whole transcriptome of endothelial cells exposed to serum from patients with COVID-19 or non-COVID-19 pneumonia.

Materials and Methods: Human umbilical vein endothelial cells (HUVECs) were treated with sera from 29 COVID-19 patients and 10 patients with non-COVID-19 pneumonia (controls). The presence of SARS-CoV-2 RNA was investigated in sera and HUVECs samples, by RT-PCR and Digital Droplet PCR (ddPCR), respectively. Transcriptomic analysis was carried out by high-throughput RNA sequencing. In addition, experiments with synthetic Protease-activated receptor 2 (PAR-2) modulators were performed by treating HUVECs in the presence of serum or PAR-2 agonist. Alterations in terms of apoptosis, thrombin generation and permeability of treated cells were examined by specific assays.

Results: COVID-19 sera induced a distinct endothelial phenotype characterized by alterations in the expression of genes of signalling pathways involved in thrombo-inflammation and apoptosis. These observations were confirmed in vitro by showing that COVID-19 sera alter functional properties of

endothelial cells leading to increased apoptosis, loss of barrier integrity, and hypercoagulability. Interestingly, the identified alterations were caused by soluble components in the sera since our analysis revealed that the sera from our cohort of COVID-19 patients and treated HUVECs cells did not contain viral RNA. PAR-2 emerged as a potential mediator of endothelial effects, as predicted by transcriptome network analysis validated by in vitro functional assays.

Discussion and Conclusions: Our findings open the way to further studies to evaluate whether targeting PAR-2 may be a clinically effective strategy to counteract endothelial dysfunction during COVID-19 disease.

115 - DEVELOPMENT OF ORAL IGA RESPONSE AGAINST SARS-COV-2 FOLLOWING IMMUNIZATION WITH DIFFERENT COVID-19 VACCINES.

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Topic: Risposta dell'ospite alle infezioni e alle vaccinazioni

Development of oral IgA response against SARS-CoV-2 following immunization with different COVID-19 vaccines.

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Introduction: Local immune response at oral level has been suggested to have an important role in the early control of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) infection at the primary site of infection and viral replication, as local secretory IgA (sIgA) were reported to inversely correlate with patients' symptom severity and oral inflammation. Moreover, sIgA were also detected in the conjunctival fluid of Coronavirus Disease (COVID)-19 patients, and SARS-CoV-2 vaccines were shown to elicit specific sIgA response at ocular level. Yet, there is still scarce information on the comparative ability of the diverse SARS-CoV-2 vaccines to induce local IgA responses at the virus entry site. Thus, the aim of this study was to characterize sIgA mucosal immunity in saliva samples collected from subjects vaccinated with a booster dose and different combinations of vaccines, including mRNA-1273 (Moderna), BNT162b2 (Pfizer-BioNTech), and Vaxzevria (AstraZeneca).

Materials and Methods: A total of 95 subjects vaccinated with different combinations of COVID-19 vaccines were recruited at the University Hospital of Ferrara (Italy) and the presence of anti-SARS-CoV-2 oral sIgA was evaluated in their saliva by a quantitative ELISA assay targeting human IgA directed against the receptor-binding domain of the viral spike protein S1-RBD (RayBiotech Life, Georgia, United States), specifically adapted for saliva assessment.

Results: The results showed the presence of a mucosal response in 93.7% of vaccinated subjects, with a mean IgA titer of 351.5 ± 31.77 U/mL, strongly correlating with the serum anti-SARS-CoV-2 IgG titer ($p < 0.0001$). No statistically significant differences emerged between the vaccine types, although the salivary IgA titer appeared higher after receiving a booster dose of the mRNA-1273 vaccine (Moderna) following two doses of BNT162b2 (Pfizer-BioNTech), compared to the other vaccine combinations, confirming previous results obtained at the eye mucosa.

Discussion and Conclusions: Monitoring the development of mucosal response in the oral cavity may drive forward vaccine design and surveillance strategies, potentially leading to novel therapeutic approaches and new routes of vaccine administration and boosting.

120 - PROPIONIBACTERIUM ACNES EXTRACT IMPROVES CELLULAR RESPONSE AGAINST CANDIDA ALBICANS AND ESCHERICHIA COLI IN IN VITRO MODELS OF VULVOVAGINAL INFECTIONS

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Propionibacterium acnes extract improves cellular response against Candida albicans and Escherichia coli in in vitro models of vulvovaginal infections

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Introduction: The Propionibacterium acnes extract (PE) is a bacterial lysate, which is included as an active compound in a gel formulation used to treat the symptoms of vulvovaginal infections. This study aims to understand and define, by an in vitro model, the PE beneficial effects upon Candida albicans (C. albicans) and Escherichia coli (E. coli) infection of vaginal epithelial cells and macrophages.
Materials and Methods: Initial studies focused on epithelial cells. The A-431 human vaginal epithelial cells were treated with PE for 24h before being infected with C. albicans or E coli. Lactate-dehydrogenase (LDH) released by damaged cells in the growth medium, as well as C. albicans growth by CFU, were evaluated. In order to assess the capacity of the PE to modulate epithelial mitochondrial activity, Reactive-Oxygen-Species (mtROS) production by the cells infected with C. albicans was measured. This parameter could be kinetically monitored through the analysis of emitted fluorescence, after the addition of the MitoSOX™ Red probe. Subsequently, the research focused on macrophages. Murine macrophages were treated with PE for 24h before being infected with C. albicans or E coli. Phagocytosis of heat-killed fluorescent C. albicans yeasts and the killing activity against C. albicans and E. coli were analyzed. Moreover, the evaluation of the mitochondrial activity was carried out, as described above.
Results: Our results show that PE treatment a) increased the mitochondrial activity of vaginal epithelial cells and macrophages in response to C. albicans infection, b) protected the epithelium against the damage induced by both pathogens, c) reduced C. albicans growth during cell infection, and d) improved phagocytosis and killing capacity of macrophages.
Discussion and Conclusions: Taken together, our results suggest that PE may improve cellular

response against both C. albicans and E. coli infection by increasing epithelial cells and macrophages activity, thus suggesting that PE may train cells to improve their response to pathogens.

128 - B- AND T- CELL-MEDIATED RESPONSE INDUCED BY THE ADJUVANTED GLYCOPROTEIN E (GE)-BASED RECOMBINANT VACCINE AGAINST HERPES ZOSTER (RZV) IN CANCER PATIENTS DURING IMMUNOTHERAPY: A ONE YEAR FOLLOW-UP STUDY

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B- and T- cell-mediated response induced by the adjuvanted glycoprotein E (gE)-based recombinant vaccine against Herpes Zoster (RZV) in cancer patients during immunotherapy: a one year follow-up study

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Background “Real-life” data on the adjuvanted glycoprotein E (gE)-based Recombinant Zoster Vaccine (RZV) in cancer patients during immune checkpoint inhibitors (ICIs) had revealed a high immunogenicity of the vaccine 28 days after the second dose. The durability of immunogenicity of RZV in cancer patients remains to be elucidated.

Patients&Methods

38 patients (median age 71 years, 73% males) were enrolled in the study. Sera and peripheral blood mononuclear cells (PBMCs) were collected at time of vaccination (T0), 4 weeks (T2), 6 (T3) and 12 months (T4) after complete RZV vaccination schedule. We were able to collect blood samples after 12 months post-vaccination in 32 (86,5%)subjects. T-cell response was evaluated by IFN-γ ELISpot assay performed against gE and IE63 peptide pools. VZV-specific IgG antibodies were quantified at each time point.

Results In the previous study, 37 patients were enrolled, while the current analyses refer to the 32 patients (10 females/22 males; median age 71 years) still on immunotherapy at the time of the follow up. Only one patient showed an asymptomatic HZ episode. The quantification of the VZV-antibodies revealed that all the patients except one were seropositive before vaccination.. A significant increase in VZV IgG titer was observed at each time point in comparison with T0.. On the other hand, peak gE

specific T-cell frequency was observed at T2 and persisted over 6 months after complete vaccination schedule. gE-specific T cell response measured, in 25 patients, 12 months post-vaccination were slightly lower than those measured at T3 (20 IQR 10.75-60.5 versus 42.5 IQR 20-120 IFN-γ-producing cells/106 PBMCs; p=0.36). Conversely, no variation was observed against IE63 peptide pool. Interestingly, only 20% of the RZV recipients showed negative gE-specific T-cell responses at 12 months post-vaccination versus 8.1% at 6 months post-vaccination indicating a long-lasting gE--specific CMI in more than two-thirds of RZV recipients.

Conclusion

In conclusion, RZV-Shingrix induced strong humoral and cell-mediated immune responses in patients with solid cancer undergoing immunotherapy. These responses persisted above pre-vaccination levels through 1 year after complete vaccination. Moreover, RZV is well-tolerated in the patients with cancer undergoing ICIs. Efficacy should be evaluated in prospective long-term studies.

129 - EX VIVO HUMAN SPLEEN PERFUSION AS A MODEL TO STUDY THE BACTERIA-MACROPHAGES INTERACTION

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Study of the bacteria-macrophages interaction through an ex vivo model of human spleen perfusion

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The bacterium Streptococcus pneumoniae is one of the major causes of serious diseases and death. Previous studies in mouse showed that invasive bacterial disease occurs after rare events of macrophage functionality failure that led to within-macrophage replication of bacteria and consequent systemic infection. S. pneumoniae is exposed to phagocyte-mediated clearance mechanisms in the spleen but the roles of the spleen macrophages in pneumococcal clearance are unknown.

To describe intracellular survival and characterize the function of human tissue macrophages, ex vivo human spleen perfusions were performed. Biopsies were taken and imaged through immunohistochemistry. The marker used during the microscopies is lamp1, aiming to correlate the bacterial clearance with macrophage lysosomes activation.

The human spleen is characterize by three different sub-populations of tissue resident macrophages. Immunohistochemistry image analysis showed that almost all the lamp1 activated-lysosome cellular marker is detected within macrophages and that the CD169+ macrophages harbour 10x more bacteria than the CD163+.

Moreover, the numbers of bacteria detected within the CD169+ decrease over time. At the same time, lamp1 was found increasing during the first 30 minutes of infections and then decreasing over time as bacterial counts do. In fact, the total spleen area covered by lamp1 decreases over time and both the numbers of CD169+ macrophages having bacteria or lamp1 decrease. Finally, T4 and 19F specific image analysis showed no significant differences between high virulent and low virulent serotypes.

Not infected and micro-beads perfused spleens showed no decrease of lamp1 over time, that the total tissue area occupied by lamp1 and the numbers of CD169+ having activated lysosomes are 10X less.

Finally, the ex vivo human spleen perfusions is allowing us a functional characterization of human tissue macrophages and showed that also in humans, replication in permissive CD169+ macrophages could be the critical point for the start of invasive infections.

133 - EFFECT OF SARS-COV-2 VACCINATION AND INFECTION ON HUMORAL AND CELLULAR IMMUNITY IN A COHORT OF PATIENTS WITH IMMUNE-MEDIATED DISEASES (IMID)

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Effect of SARS-COV-2 vaccination and infection on humoral and cellular immunity in a cohort of patients with immune-mediated diseases (IMID)

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Abstract:

Aim of the study: Immunization against COVID-19 is needed in patients with immune-mediated inflammatory diseases (IMIDs). However, data on long-term immunity kinetics remain scarce. The study aimed to compare the humoral and cellular response to COVID-19 in patients with immune-mediated inflammatory diseases (IMIDs) compared to healthy controls. We compared the humoral and cellular response to SARS-Cov-2 elicited by vaccination and/or infection in a prospective cohort of IMID patients compared with a group of health care workers (HCWs). Methods: We assessed immunity before and after the third and fourth dose of BNT162b2 or after COVID-19 infection using quantitative IgG anti-SARS-CoV-2 Spike antibody (anti-S-IgG), neutralization assay, and specific interferon-gamma (IFN-g) release assay (IGRA). The responses were compared with those of healthy controls. Results and conclusions: The two groups were similar in age and total exposure, becoming infected for the first time, mainly after the third dose. Neutralizing antibodies and IGRA were negative in 9.5% of IMID but not in any HCWs. No significant difference was found between neutralization titers to BA.1 in the IMID and the HCWs group. The study highlights the SARS-CoV-2 immunological responses in healthy

controls and IMID patients, suggesting that the combined stimuli of vaccination and infection in IMID patients could promote a more profound immunological response.

143 - SEX INFLUENCE ON THE CYTOKINES PRODUCTION BY AMNIOTIC FLUID CELLS UPON MYCOPLASMA HOMINIS INFECTION

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Sex influence on the cytokines production by amniotic fluid cells upon Mycoplasma hominis infection

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Introduction: Mycoplasma hominis is a human obligate parasite capable to colonize the cervix or vagina of adult women. Since their small size, mycoplasmas can cross the placental membranes and colonize amniotic fluid, triggering the preterm pre-labour rupture of membranes, spontaneous abortion, and preterm birth. Several studies have demonstrated that foetal sex is a risk factor for preterm birth, with a higher risk for male foetuses than females. Moreover, the sex differences affect the inflammatory response, with more active immune responses in females than in males linked with higher pro-inflammatory cytokines secretion. These molecules are crucial in the host defence against pathogens but so far, there are no data regarding the influence of foetal sex on cytokines production upon bacterial infections during pregnancy. This study aimed to investigate the response of cells from the amniotic fluid (AFC) of male and female foetuses in mid-pregnancy upon infection with M.hominis, by measuring IL6, IL8, and TNF-alpha gene and protein, as toll-like receptor 4 (TLR4) mRNA levels.

Materials and Methods: AFCs were infected with M.hominis culture for 24 hours and supernatants were collected and analysed by ELISA to evaluate the IL6, IL8, and TNF-alpha concentration. RNA was extracted from AFCs to analyse gene expressions of cytokines and TLR4. AFCs not infected were used as negative controls.

Results: M.hominis infection led to a significant increase in the expression of IL6, IL8, and TLR4 genes, but not of TNF-alfa if compared to basal conditions, with specific sex differences. In particular, IL6, IL8, and TNF-alfa mRNA levels were significantly higher in female AFCs than males. These results were supported by ELISA data: IL6 and TNF-alfa concentration in supernatants was significantly higher in female AFCs than in basal conditions and male AFCs. IL8 did not show any difference.

Discussion and Conclusions: Our results showed that female foetuses exhibited greater stimulated cytokines production during the mid-trimester of pregnancy than male foetuses, suggesting that differential inflammatory responses could affect maternal health and foetal development, predisposing to sex-specific foetal programming. The foetal sex could help to diagnose risk predictions of pregnancy outcomes and prevent future disease in adulthood.

150 - MIR-182-5P AND MIR-221-3P AS POTENTIAL REGULATORS OF HERV-K EXPRESSION IN AMYOTROPHIC LATERAL SCLEROSIS

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MiR-182-5p and miR-221-3p as Potential Regulators of HERV-K Expression in Amyotrophic Lateral Sclerosis

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Abstract

Introduction. Amyotrophic lateral sclerosis (ALS) is a highly debilitating neurodegenerative disease for which there is no treatment. It is classified as a multifactorial disease, as various genetic and environmental factors contribute to its onset and progression. Recent studies have highlighted the role of Human Endogenous Retroviruses (HERVs) in the etiopathogenesis of ALS, with particular emphasis on the most active and recently integrated, HERV-K. In particular, reactivation of HERV-K under pathological conditions has been documented to negatively affect disease progression. However, the mechanisms through which this reactivation occurs remain unclear. In the present study, our goal is to explore the potential role of microRNAs (miRNAs) in modulating the expression of HERV-K.

Materials and methods. The identification of miRNAs capable of binding to the HERV-K transcript was conducted using the online tool miRDB. Among the microRNAs identified, we specifically focused on miR-182-5p and miR-221-3p, both of which are implicated in neurodegenerative processes. Experiments to investigate the binding interactions between these microRNAs and the consensus sequence of HERV-K were carried out in HEK-293 cells. These experiments involved co-transfection with miRNA and HERV-K consensus sequence, followed by quantitative real-time-qPCR to quantify gene expression levels of HERVs. Additionally, Western blotting assays were conducted to assess the protein levels. To validate our findings, real-time-qPCR was performed on a cohort consisting of ALS patients and healthy controls. This allowed for the determination of expression levels of both HERV-K and the microRNAs.

Results. Our results from HEK-293 cell co-transfection have demonstrated that these microRNAs are capable of binding to the transcript of HERV-K. This interaction results in a significant downregulation of the expression of its genes, with a pronounced effect observed on the envelope gene. Subsequently, we validated these findings in a population of ALS patients compared to healthy individuals. Our results show that HERV-K-envelope levels are elevated in ALS patients compared to controls ($p = 0.02$) and these levels negatively correlate with the gene expression levels of the microRNAs, which are downregulated in patients versus healthy controls (miR-182-5p, $p = 0.02$; miR-221-3p, $p = 0.009$).

Discussion and conclusions. Our results highlight a potential relationship between microRNA expression levels and HERV-K expression levels, supporting our hypothesis that the downregulation of key microRNAs may lead to deregulated HERV-K expression. However, further studies are required to confirm these interactions and to understand their implications in the pathophysiology of ALS.

163 - INNATE IMMUNE CELLS RESPONSE TO RESPIRATORY VIRUSES

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Topic: Risposta dell'ospite alle infezioni e alle vaccinazioni

Title: INNATE IMMUNE CELLS RESPONSE TO RESPIRATORY VIRUSES

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Introduction

During infections, the principal actors of the immune innate system are the antigens presenting cells and the released stimuli of cytokines and chemokines.

Our aim is to analyse the effect on monocytes an monocyte derived macrophages driven by different respiratory viruses (SARS-CoV-2 VOCs, FluA H1N1 and H3N2, FluB, OC43, MPV), and define the role of innate immune cells in viral dissemination.

Materials and Methods

Several respiratory viruses were isolated from PCR positive nasopharyngeal swabs. Each virus was propagated and titrated using permissive cells (VERO E6 for SARS-CoV-2, MDCK for FluA and FluB, LLC-MK2 for MPV and for OC43 were used both VEROE6 overexpressing TMPRSS2 and human derived fibroblasts). Monocytes (MN) were isolated from peripheral blood mononuclear cells from healthy donors and differentiated in monocytes derived macrophages (MDMs). Evaluation of MDMs infection at various time points was performed by IFA, RT-qPCR (intra/extracellular) and viral titration. To assess viral transmission, permissive cells were infected with each respiratory virus and cocultured with uninfected MDMs. Then, MDMs ability to migrate in response to chemoattractants was exploited. Migrated MDMs were cocultured with naïve permissive cells to assess viral dissemination by cytopathic effect development and IFA.

Results

Time-course experiments show that SARS-CoV-2 VOCs persist, but do not replicate inside macrophages. There is no increase in the viral load both in the supernatant or intracellular, while in VERO E6 cells the viral loads highly increase. Nonetheless, while in co-colture macrophages can transmit the SARS-CoV-2 VOCs to permissive VERO E6. Similarly to SARS-CoV-2 VOCs, the other respiratory viruses used in this work are also internalized by macrophages.

Discussion and Conclusions

Infected cells with active viral replication transmit the virus to macrophages, where the virus persists but does not replicate. Infected macrophages can transmit the virus to permissive cells. Therefore, these immune cells can host viable virus but are not permissive to viral replication. These in vitro observations suggest that macrophages are key players in viral dissemination and persistence.

164 - EX-VIVO SPLEEN PERFUSION: GM-CSF PRODUCTION BY MACROPHAGES DURING STREPTOCOCCUS PNEUMONIAE INFECTION

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Ex-vivo spleen perfusion: GM-CSF production by macrophages during Streptococcus pneumoniae infection

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Introduction

Splenic tissue macrophages are key immune cells in clearing bacteria from the blood.

Streptococcus pneumoniae is one of the major causes of community acquired pneumonia, which can progress to sepsis, and is exposed to phagocyte-mediated clearance mechanisms in the spleen. To begin deepening our knowledge on the dynamics of macrophages-pneumococci interactions, we focused on GM-CSF (Granulocyte-macrophage colony-stimulating factor). GM-CSF is a cytokine produced by different immune cells, including macrophages, and regulates inflammatory responses.

Materials and methods

Ex-vivo perfused human spleens were infected with a mix of different pneumococcal serotypes for six hours. Biopsies taken at different time-points stained for bacteria, macrophages and GM-CSF and analyzed through microscopy.

Results

Analysis of the immunohistochemistry images focused on understanding whether the two different splenic populations (CD163+ and CD169+) display differences in bacteria association and GM-CSF expression. CD163+ and CD169+ macrophages showed similar bacteria association throughout the infection. CD163+ displayed an increase in GM-CSF production during the infection. CD169+

macrophages, on the other hand, showed higher levels of GM-CSF in early times, and decreased at later times.

Discussion and Conclusions

In conclusion, the ex vivo perfusion of human spleens is a valuable model to functionally characterize human tissue macrophages. The data obtained provide some hints on the role of GM-CSF production by macrophages during the pneumococcal infection.

165 - GALLERIA MELLONELLA LARVAE AS AN INNOVATIVE TOOL FOR IMMUNE PROFILING, INFECTION STUDIES AND DRUG SCREENING.

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Galleria mellonella larvae as an innovative tool for immune profiling, infection studies and drug screening

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INTRODUCTION: Galleria mellonella larvae represent an alternative animal model due to their ease of use, the absence of ethical rules and their analogy with the mammalian immune system. In order to develop the full potential of this model, we present a novel approach to characterise the haemocyte population of G. mellonella larvae highlighting the immune modulation following infection and treatment.

MATERIALS AND METHODS: G.mellonella larvae at their sixth-stage, weighing 200-250 mg, were selected for the experiments. Animals were divided in experimental groups to be infected with different concentrations of S.aureus ATCC43300 suspension in PBS; 30 min after the infection, larvae were randomized to receive various dosages of vancomycin (V). Appropriate controls were included. At different time points after the injections (0-3-24h), the hemolymph of the larvae was harvested. Infected haemocytes derived from G. mellonella were subjected to immunophenotype analysis at set times (time 0, 3 and 24 hours) by incubating the cells with anti-CD (differentiation clusters) 14-44-80-163-200 human. In addition, haemocytes were isolated and subcultured up to 24 hours and the cell morphology was analysed.

RESULTS: The concentration of 106 CFU/larva of S.aures ATCC43300 replicates the main infection scenario. Vancomycin demonstrated a dose-dependent trend, therefore the dose of 50mg/kg was selected to achieve the best healing conditions. G. mellonella isolated haemocytes expressed cell membrane markers typically expressed by human immune cells following inflammation and infection

such as CD14, CD44, CD80, CD163 and CD200. Furthermore, morphological differences within the hemocytes population were appreciated.

DISCUSSION AND CONCLUSION: Our new approach confirms that invertebrates and vertebrates share evolutionarily conserved components among innate immune responses: the hemocytes from G.mellonella react with anti-human antibodies directed against the markers CD14, CD44, CD80, CD163, and CD200 commonly used for in vitro immunophenotyping. The analysis also reveals that the population is highly heterogeneous and the immunophenotypic profile significantly different from that of a homogeneous monocytic/macrophage cell line. G.mellonella hemocytes are highly responsive to inflammatory infections and their immunophenotype is modulated by drugs in parallel, as occurs in human blood monocytes. Although with limitations related to the use of anti-human antibodies to discriminate hemocytes from an invertebrate, the data obtained demonstrated that this model may represent a suitable tool for screening vaccines as well as new compounds that act as immunomodulators or antibiotics.

192 - PHENOTYPIC MODULATION OF SPIKE-SPECIFIC B CELLS IN PEOPLE LIVING WITH HIV UPON BOOSTER VACCINATION WITH SARS-COV-2 MRNA VACCINES

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Phenotypic modulation of spike-specific B cells in people living with HIV upon booster vaccination with SARS-CoV-2 mRNA vaccines

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Introduction. People living with HIV (PLWH) exhibit reduced immune memory persistence to numerous vaccine antigens. In the context of recent COVID-19 pandemic, SARS-CoV-2 mRNA vaccines have proved to be immunogenic in PLWH under antiretroviral therapy. However, most studies have focused on the serological response, while data on the generation and persistence of spike-specific memory B cells (MBC) are limited. To this aim, we have characterized how booster doses impact on the quantity and quality of the spike-specific MBC response.

Materials and Methods. Spike-specific humoral and memory B cell responses were investigated up to 2 years after first mRNA vaccination in 115 PLWH under antiretroviral therapy and compared to 85 healthy controls (HCs).

Results. The frequency of spike-specific MBC increased upon the second and the third vaccine dose, while a weaker booster effect was observed upon the fourth dose. MBC persisted 2 years after vaccination, and their phenotype was modulated by vaccine doses administration. Indeed, resting MBC (CD21+CD27+) increased with vaccine administration. While a consistent frequency of DN cells (IgD-CD27-) was observed upon the second dose, this subset was reduced after booster dose. Spike-specific IgG were still present at year 2 in PLWH, but at lower titers compared to HCs.

Discussion and conclusions. SARS-CoV-2 mRNA vaccines elicit long-term immune memory response in PLWH and booster doses impact on the magnitude and phenotype of antigen-specific MBC. These findings provide valuable direction for improving tailored vaccination for PLWH.

203 - DUAL ROLES OF MIR-9 IN MODULATING NF-KB SIGNALING AND CYTOKINES EXPRESSION IN COVID-19 PATIENTS

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Dual Roles of miR-9 in Modulating NF-kB Signaling and Cytokines Expression in COVID-19 Patients

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Abstract:

Introduction: The severe manifestations of COVID-19 are significantly influenced by dysregulated immune responses, particularly through the NF-kB signaling pathway. The microRNA miR-9 has been identified as a regulator of this pathway, affecting both the activation of NF-kB and the expression of crucial pro-inflammatory cytokines. Understanding miR-9's modulatory role is essential for identifying potential biomarkers and therapeutic targets for managing severe COVID-19 outcomes.

Materials and Methods: This retrospective study included 41 COVID-19 patients admitted during the first pandemic wave (March-June 2020) at the University Hospital Tor Vergata in Rome, Italy, along with 20 age and sex-matched healthy controls. The expression levels of miR-9, NF-kB, and IκBα, as well as the pro-inflammatory cytokines IL-6, IL-1β, and TNFα were quantified using reverse transcription-quantitative PCR (RT-qPCR). A comprehensive statistical analysis, including linear regression models, was employed to examine the correlations between miR-9 expression and inflammatory markers.

Results: Elevated levels of miR-9 were observed in non-surviving COVID-19 patients compared to survivors and healthy controls, with a significant increase in NF-kB expression also noted in the patient cohort. IκBα was undetectable in all patient samples, suggesting a disruption in NF-kB regulatory controls. The correlation analysis revealed that miR-9 expression positively correlated with TNF-α levels ($\beta = 2.506$, $P = 0.047$) and negatively correlated with IL-6 ($\beta = -0.622$, $P = 0.033$) and IL-1β ($\beta = -0.292$, $P = 0.021$). These findings indicate a nuanced role for miR-9 in influencing the inflammatory profile of COVID-19 patients, showing both enhancement and suppression of different cytokine pathways.

Discussion: The study underscores the dualistic influence of miR-9 on the inflammatory response in COVID-19, capable of both upregulating and downregulating crucial cytokines through distinct mechanisms. The overexpression of NF-kB in the absence of its inhibitor IκBα highlights a potential mechanism underlying the severe cytokine storm observed in critical COVID-19 cases. Additionally, the differential regulation of cytokines by miR-9 suggests its role in fine-tuning the immune response, which may have significant implications for both disease progression and recovery.

Conclusions: miR-9 significantly modulates inflammatory responses in COVID-19 by regulating NF-kB and its target cytokines. These interactions suggest that miR-9 could be a promising biomarker and therapeutic target for controlling inflammation in SARS-CoV-2 infection. Further research is required to elucidate the complete regulatory mechanisms of miR-9 and its potential therapeutic benefits in viral infections.

207 - ANTI-SPIKE ANTIBODY RESPONSE AND IFN SIGNATURE IN PEOPLE LIVING WITH HIV RECEIVING SARS-COV-2 MRNA-BASED VACCINE: A LONGITUDINAL STUDY

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Anti-Spike antibody response and IFN Signature in People Living with HIV Receiving SARS-CoV-2 mRNA-based Vaccine: A Longitudinal Study

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Introduction

Published data demonstrate that the mRNA-based SARS-CoV-2 vaccine elicits humoral and cellular immunity in HIV-1 individuals. However, the impact of the innate immune response on the efficacy of COVID-19 vaccination in people living with HIV (PLWH) over time remains to be defined. Therefore, we aimed to analyze the anti-Spike antibody response and type I Interferon (IFN-I) signature in PLWH receiving COVID-19 vaccine over a one-year longitudinal study.

Material and Methods

Blood samples were collected from HAART-treated PLWH (n=75) at baseline (prior to SARS-CoV-2 vaccination, T0), at the time of the second (2nd) dose (T1), 1 (n=48) or 6 months (n=27) after the 2nd dose (T2) and 1 year after the 2nd dose (T3). Measurement of SARS-CoV-2 Trimeric IgG was performed by chemiluminescent immunoassay. The levels of anti-SARS-CoV-2 IgG were compared to a group of healthy donors (HD, n=28) at each time point. Gene expression of IFN-I (IFN-alpha, IFN-beta and IFN-omega) was measured by RT/Real Time PCR in PBMC from 56 patients.

Results

Anti-SARS-CoV-2 Trimeric IgG levels increased significantly at T1 compared to T0, and at T2 compared to T1 ($p<0.001$), and there was a trend toward an increase at T3 in comparison to T2. HDs showed the same trend of a longitudinal increase in anti-SARS-CoV-2 Trimeric IgG (T0 vs T1 and T2 vs T3 $p<0.001$). No significant differences were observed in anti-SARS-CoV-2 Trimeric IgG levels between PLWH and HDs. The baseline gene expression of IFN-alpha, IFN-beta and IFN-omega does not affect the development of anti-S IgG at any time point analyzed. Multivariate analysis of type I IFN transcript levels over time revealed that IFN-alpha, IFN-beta, and IFN-omega gene expression was comparable at each time point analyzed.

Discussion and Conclusions

We found that PLWH showed an antibody response after SARS-CoV-2 vaccination comparable to that observed in healthy donors, which was not influenced by IFN levels. Furthermore, vaccinating against COVID-19 does not interfere with the IFN-I signature.

215 - PREDICTING HUMORAL RESPONSES TO PRIMARY AND BOOSTER SARS-COV-2 MRNA VACCINATION IN PEOPLE LIVING WITH HIV: A MACHINE LEARNING APPROACH

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Predicting humoral responses to primary and booster SARS-CoV-2 mRNA vaccination in people living with HIV: a Machine Learning approach

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Keywords: Machine Learning, SARS-CoV-2, HIV, Statistical Modeling, Vaccines, mRNA, antibodies, Immune Response, ImmunoVirology

Abstract

Background: SARS-CoV-2 mRNA vaccines are highly immunogenic in people living with HIV (PLWH) on effective antiretroviral therapy (ART). However, whether viro-immunologic parameters or other factors affect immune responses to vaccination is debated. This study aimed to develop a Machine Learning (ML)-based model able to predict the humoral response to mRNA vaccines in PLWH and to assess the impact of demographic and clinical variables on antibody production overtime.

Methods: Different ML-algorithms have been compared in the setting of a longitudinal observational study involving 497 PLWH, after primary and booster vaccination. Both Generalized Linear Models and non-linear Models (Tree Regression and Random Forest [RF]) were trained and tested.

Results: Non-linear algorithms showed better ability to predict vaccine-elicited humoral responses. The best-performing RF model identified a few variables as more influential, within 39 clinical, demographic, and immunological factors. In particular, previous SARS-CoV-2 infection, BMI, CD4 T-cell count and CD4/CD8 ratio were positively associated with the primary cycle immunogenicity, yet their predictive value diminished with the administration of booster doses.

Conclusions: In the present work we have built a non-linear RF ML model capable of accurately predicting humoral responses to SARS-CoV-2 mRNA vaccination, and identifying relevant factors that influence the vaccine response in PLWH. In clinical contexts, the application of this model provides promising opportunities for predicting individual vaccine responses, thus facilitating the development of vaccination strategies tailored for PLWH.

233 - ANTIGEN-SPECIFIC T CELL RESPONSE IN TRANSPLANT RECIPIENTS AFTER HUMAN CYTOMEGALOVIRUS INFECTION

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Antigen-specific T cell response in transplant recipients after Human Cytomegalovirus infection

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Abstract

Introduction. Human Cytomegalovirus (HCMV) infection remains a major complication in transplanted patients. After infection, the recovery of T cell response is an indicator of protection from HCMV disease. In this study, HCMV-specific CD4+ and CD8+ T cell response and their cytokine production (IFN-γ, TNF-α, IL-2), against different HCMV proteins (IE-1, pp65, gB, gH/gL/pUL128L), was analyzed in solid organ transplant recipients (SOTR) and hematopoietic stem cell transplant (HSCT) patients with infection.

Materials and Methods. Twenty SOTR (14 kidney, 3 hearth and 3 lung) and sixteen HSCT were clustered in two groups: i) patients that controlled spontaneously the HCMV infection (controllers) and did not require antiviral therapy and ii) patients that did not control the infection (non-controllers) and needed antiviral therapy. The peptide-specific T cell response was investigated after stimulation with peptide pools of the selected antigens and analyzed with intracellular staining (ICS) and Cytokine Flow Cytometer (CFC). The pentameric complex gH/gL/pUL128L was divided into gH and gL/pUL128L pools.

Results. All patients developed a higher CD4+ IFN-gamma+ HCMV-specific T cell response towards pp65 and IE-1 peptides at the peak of the infection, to arrive at the resolution of the infection with an almost equal specificity for all peptide pools, with the exception of pUL128L which was slightly lower. As far as CD8+ IFN-gamma+ HCMV-specific T cell response, it was higher for pp65 and IE-1 compared to gB, gH and pUL128 at the peak and at the resolution of the infection, for both controllers and non-controllers.

However, the frequency of HCMV-specific T cell response, for both CD4+ and CD8+, against pp65 was higher in controllers vs non-controllers patients. Higher frequency of mono-functional, producing only IFN-gamma, and bi-functional producing IFN-gamma/TNF-alfa pp65-specific T-cell response was observed in controllers compared to non-controllers patients.

Conclusions. Higher pp65-specific CD4+IFN-gamma + and CD8+IFN-gamma + T cell response was observed in patients who controlled the infection in comparison with those who did not. The use of the pp65 peptide and the production of IFN-gamma could represent two important parameters for identifying patients who are able to control HCMV infection.

235 - AIEC-DEPENDENT PTH17 CELLS GENERATION RELIES ON DISTINCT VIRULENCE-DETERMINANTS IMPLICATED IN PERSISTENCE AND INFLAMMATORY RESPONSE OF INTESTINAL EPITHELIAL CELLS AND MACROPHAGES

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AIEC-dependent pTh17 cells generation relies on distinct virulence-determinants implicated in persistence and inflammatory response of intestinal epithelial cells and macrophages

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Introduction: Crohn's disease (CD) is a chronic inflammatory bowel disease that has been link to a strong expansion of pathogenic IFNg-producing Th17 (pTh17) cells and a mucosal enrichment of the adherent-invasive E. coli (AIEC) pathotype. In particular, we demonstrated that chronic intracellular persistence of AIEC within dendritic cells (DCs) triggers the massive IL-23 secretion and drives the generation of the IFNg-producing pTh17 cells selectively in CD patients.

So far, AIEC virulence is mainly defined at the phenotypic level for its ability to adhere and invade intestinal epithelial cells (IECs), together with its proficiency in persisting within macrophages. Conversely, whether these AIEC-virulence determinants also promote intracellular persistence within DCs and pTh17 cell generation is still unknown.

Materials and Methods: Starting to the AIEC virulence-determinants promoting intracellular persistence and triggering the detrimental inflammatory response by IECs and macrophages (fimH, ibeA and htrA), we generated isogenic deletion mutants in the AIEC reference strain LF82. The probiotic E. coli Nissle 1917 (EcN) strain was used as iso-phylogroup non-pathogenic control. Infection assays to analyze the proficiency of AIEC-mutants to persist within DCs derived from healthy donors (HD) and CD patients have been performed. Polarizing inflammatory response of AIEC-infected DCs and the antigen-specificity assay for pTh17 cell trans-differentiation were also analyzed.

Results: No difference either in the uptake or in the intracellular persistence comparing isogenic deletion mutants in fimH ibeA and htrA genes and LF82 parental strain was observed, irrespective of the DCs origin. All mutants triggered a significant higher inflammatory response in DCs derived from CD patients compared to HD. Of note, only fimH mutant displayed a lower IL-23 secretion by DCs similar to the inflammatory response triggered by EcN. In contrast, none of the AIEC-mutants tested hampered the transdifferentiation of cTh17 in pTh17 cells.

Discussion and Conclusions: Albeit ibeA and htrA are necessary for intracellular persistence within human macrophages, they were not involved either in the DCs-intracellular persistence or in the DC-dependent pTh17 cells generation. Conversely, fimH is essential for AIEC invasion/intracellular persistence within IECs, but its deletion fails to block the skewing of cTh17 into pTh17 cells. Therefore, AIEC-dependent DCs inflammatory response and pTh17 cell generation rely on distinct AIEC virulence-determinants. The identification of these AIEC-virulence determinants could pave the way for the development of new therapeutic strategies able to prevent the exaggerated inflammation and tissue damage in CD patients.

236 - CLINICAL P. AERUGINOSA STRAINS PROMOTE GENERATION OF PATHOGENIC TH1/17 CELLS THROUGH A SUSTAINED INTRACELLULAR PERSISTENCE AND POLARIZING INFLAMMATORY RESPONSE OF DENDRITIC CELLS

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Clinical P. aeruginosa strains promote generation of pathogenic Th1/17 cells through a sustained intracellular persistence and polarizing inflammatory response of dendritic cells

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Introduction: Cystic Fibrosis (CF) is a genetic disease characterized by chronic and exaggerated inflammatory response to persistent bacterial lung infections. P. aeruginosa (Pa) has been pointed out as one of the most concerning determinants in CF pathogenesis due to its ability to adapt to the CF lung environment, finally leading to generation of persistent pathoadaptive variants able to promote a sustained activation of the innate and adaptive immune system. In this regard, besides the detrimental pro-inflammatory role of neutrophils and macrophages, IL-17/IFNgamma co-producing Th1/17 cells seem to have a cardinal pathogenic role in CF. Nevertheless, the link between Pa infection and generation of lung-infiltrating Th1/17 cells has never been characterized.

Materials and methods: The frequencies of Th1/17 cell subsets in blood and lung of CF patients have been analysed by flowcytometry. Transcriptome profiles of lung-infiltrating Th1/17 cell subsets, purified from CF and non-CF patients, were also analysed by RNA-Seq. An antigen-dependent functional assay was employed to evaluate whether Pa was directly implicated in the trans-

differentiation process of ex-vivo isolated human conventional Th17 cells (cTh17) into pathogenic Th1/17 cells.

Results: Among lung-infiltrating lymphocytes, CCR5+Th1/17 cells were significantly and selectively enriched in the lungs of CF patients chronically infected with Pa. In contrast, Th1 cells were significantly decreased while cTh17 cells did not change among analysed patients. Comparative analysis of transcriptomic profiles revealed distinct and specific molecular signatures of lung-infiltrating CCR5+Th1/17 cells isolated from CF lungs. Clinical Pa strains resulted extremely proficient in persisting within human dendritic cells (DCs) as compared to laboratory strains. Moreover, pathoadaptive variants persisted significantly more than their clonal strain isolated at the early phase of the disease. Of note, both early and late variants triggered a significant higher release of IL-1beta, but not of IL-23, linked to Th1/17 cell differentiation, as compared to laboratory strains. Finally, the skewing of pathogenic Th1/17 cells from cTh17 cells was strongly promoted by some clinical Pa strains, with no significant difference between early and late variants.

Discussion and conclusions: Pa clinical strains chronically persist within DCs promoting a massive generation of pathogenic Th1/17 cells. Deciphering the molecular mechanisms behind Pa-dependent generation of Th1/17 cells could lead to the identification of crucial bacterial and immunological targets that could be used for the development of new and more efficient therapeutic strategies in CF.

247 - THE MULTIPARAMETRIC PROFILE CHARACTERIZED BY HUMAN ENDOGENOUS RETROVIRUSES, IMMUNOLOGICAL AND CLINICAL PARAMETERS DEFINES THE HETEROGENEITY OF COVID-19 AND LONG COVID SEQUELAE

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Title: The multiparametric profile characterized by human endogenous retroviruses, immunological and clinical parameters defines the heterogeneity of COVID-19 and long COVID sequelae

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Introduction: It is already known the ability of infectious agents to activate Human Endogenous Retroviruses (HERVs). We have previously demonstrated HERVs implications during SARS-CoV-2 infection and in COVID-19. Considering the complex alterations of the immune response in the pathogenesis of COVID-19 and the persistence of this dysregulation in individuals with Long COVID (LC), the aim of this study was the evaluation of HERVs in the immune pathogenesis of COVID-19 and LC. Materials and Methods: Blood samples were collected at Tor Vergata University Hospital of Rome from Acute COVID-19 patients (COV) and Healthy Donors (HD). Blood from LC individuals with post-acute sequelae of SARS- CoV-2 was also collected. The expression of the envelope (ENV) of pHERV-W and HERV-K has been analysed by Real Time and flow cytometry (FC). The T cell differentiation markers were analysed by FC and the circulating cytokines by ELLA. To identify associations between biomarkers in a multivariate manner, a factor analysis followed by varimax rotation and Kaiser normalization was performed (Principal Component Analysis, PCA). Results: ENV of pHERV-W and HERV-K have been found higher in blood samples from COV with respect to HD, and even after several weeks after infection the expression remained elevated in LC. In COVID-19, deregulation of markers of T-cell differentiation and functional depletion, circulating pro-inflammatory cytokines and vascular damage proteins, as well as biochemical parameters associated with coagulation was found. In LC, despite a restore of the normal distribution of leukocytes subpopulation, the percentage of CD4 naïve cells and CD8 terminal effector memory resulted altered and associated to inflammation, vascular

damage and coagulation, highlighting persistent immune dysfunction associated to long-term health problems. In COVID-19, PCA analysis showed different expression profiles of HERVs associated with markers of T-cell dysfunction, pro-inflammatory cytokines and vascular damage and of coagulation. Notably, in LC, PCA analysis described a specific a HERVs associated multi-parametric profile found modified in patients with specific psychiatric and neurological symptoms suggesting their involvement in neurological alterations related to COVID-19. Discussion and Conclusion: These data suggest an implication of HERVs with immune dysfunction and long-term complications of COVID-19 and candidate HERVs as biomarkers for personalized medicine.

Funded by HERVCOV project (GA101057302) HORIZON-HLTH-2021-DISEASE-04 Personalised medicine and infectious diseases: understanding the individual host response to viruses.

249 - HYBRID IMMUNITY ASSESSMENT AND ASYMPTOMATIC INFECTION RECOGNITION DURING THE SARS-COV-2 VACCINATION CAMPAIGN THROUGH MACHINE LEARNING APPROACHES

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Hybrid immunity assessment and asymptomatic infection recognition during the SARS-COV-2 vaccination campaign through machine learning approaches

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Introduction Assessing immunity elicited only by vaccination against SARS-CoV-2 has become more complex after the emergence of high transmissible viral variants. This has led to the emergence of “hybrid immunity”, a state where immunity arises from both vaccination and natural infection. Despite serological response against the Nucleocapsid (N) protein is commonly used for tracking natural infections, anti-N antibodies have limited persistence over time and a percentage of individuals remain seronegative even shortly after infection. Here, we developed a machine learning model to identify subjects who may have experienced asymptomatic infection and distinguish hybrid from vaccination immunity.

Materials and Methods Blood samples were collected from 116 subjects vaccinated against SARS-CoV-2 (IMMUNOCOV clinical study, Siena University Hospital) 6 months after the third vaccine dose. Samples were analysed by ELISA for IgG targeting Spike protein from wild type (wt), Delta, Omicron BA.1 and BA.2 variants and by surrogate virus neutralization test against the wt and the Omicron BA.2 variants. Memory B cells (MBCs) specific for wt RBD were analysed by flow cytometry, and the MBCs in vitro reactivation following stimulation with wt Spike, RBD and N antigens was assessed by ELISPOT. Data analysis and modelling utilized the UMAP dimensionality reduction technique and both Gaussian Mixture Model (GMM) and k-Nearest Neighbour (k-NN) clustering algorithms. SARS-CoV-2 infection was self-reported by participants in a survey.

Results Based on serological data, the GMM identified a cluster of high- (HRs) and of low responders (LRs). Most infected subjects were classified as HRs, though the impact of infection on this categorization waned after few months. A k-NN model, built on serological data, was developed to discriminate between non-infected subjects, those who may have had asymptomatic infections and infected individuals. The model demonstrated an average accuracy of 0.98 and an average precision of 0.97 during cross-validation on a subset of our cohort. Applied to the rest of the cohort, the model correctly recognized 85% of self-declared infected subjects and identified 14 subjects without prior

infection history showing serological profiles similar to vaccinated and infected individuals. These subjects, who may have had asymptomatic infection, and infected subjects also displayed similar frequency of RBD-specific memory B cells with a prevalent IgG+ resting memory phenotype, higher than non-infected subjects.

Discussion and Conclusions To conclude, we developed a machine learning model based on serological data capable to identify subjects that have experienced a suspected asymptomatic infection and to discriminate vaccine-induced from hybrid immunity.

251 - A MURINE MODEL OF INVASIVE ENTERIC INFECTION WITH SALMONELLA ENTERICA SEROVAR TYPHIMURIUM AS A TOOL TO STUDY HOST RESPONSE TO INFECTION AND VACCINE PROTECTION

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A murine model of invasive enteric infection with Salmonella enterica serovar Typhimurium as a tool to study host response to infection and vaccine protection

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Introduction. Invasive Nontyphoidal Salmonella (iNTS) infection is a major cause of death in sub-Saharan Africa, and currently no licensed vaccine against iNTS is available. Salmonella enterica serovar Typhimurium (S. Typhimurium) is an invasive species that generally causes gastroenteritis in immunocompetent individuals. However, in immunocompromised subjects, it is often associated with invasive infection such as septicaemia and/or meningitis with no or limited enteric symptoms. Novel or improved animal models that closely mimic human infection are needed to study in vivo iNTS pathogenesis as well as host responses to infection and vaccine induced protection. In the present study, a murine model of enteric infection using the well-known iNTS clinical isolate D23580 (ST313) was developed and used as a tool to characterize the immune response and protection induced by a model vaccine against iNTS.

Materials and Methods. C57BL/6 susceptible mice were vaccinated with 3 doses (10^7 colony forming units (cfu)/mouse) of formalin-inactivated S. Typhimurium D23580 strain and challenged 10 weeks later by the intragastric route (i.g.) without pretreatment with streptomycin in order not to perturb the intestinal mouse microbiota. Mice were infected i.g. with 10^7 cfu/mouse of the D23580 strain and daily monitored. Survival analysis was supported by daily recording of clinical parameters (body weight and clinical score). S. Typhimurium-specific IgG induced by vaccination was analysed in serum at different time points. Bacterial dissemination in blood, spleen, liver, and bacterial shedding through faeces were assessed 6 days post-infection (dpi) in SS agar plates with (for faecal samples) or without (for sterile sites) antibiotic selection.

Results. Survival rate of mice vaccinated and infected with the D23580 iNTS isolate was 100% up to 6 dpi. Between 6 and 9 dpi, survival rate dropped to 50% and then remained stable. On the contrary, survival of control infected animals gradually decreased from 6 to 15 dpi, when no mice were alive after challenge. Results on body weight and clinical scores of infected mice were consistent with

survival data. Six dpi S. Typhimurium dissemination in blood and liver and shedding in the faeces were significantly reduced in vaccinated and infected animals compared to infected controls.

Discussion and Conclusions. In the present study, we have set up and employed a mouse model of iNTS disease that closely mimics the invasive infection in humans to investigate immunity and protection induced by a model vaccine against iNTS. The present model represents a valuable tool to study host-pathogen interactions, host response to infection and evaluate protection conferred by vaccine candidates against iNTS.

254 - INSIGHT INTO SARS-COV-2 BREAKTHROUGH INFECTIONS: SEROLOGICAL AND VIROLOGICAL ASPECTS

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Insight into SARS-CoV-2 Breakthrough Infections: Serological and Virological aspects

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Introduction

Despite the high level of vaccine efficacy, a percentage of vaccinated individuals develop symptomatic or asymptomatic SARS-CoV-2 infections, defined as breakthrough infections. Some breakthrough infections are likely due to the emergence of viral variants that evade the immunity developed through vaccination or to the lack of response to vaccination. The aim of this study was to describe breakthrough infections in patients at high-risk of developing severe COVID-19 from both an immunological and virological perspective.

Materials and Methods

From March 2021 to May 2022, 177 high-risk patients with mild-moderate COVID-19 were recruited for treatment with monoclonal antibodies (mAbs). Nasopharyngeal swabs (NPS) and plasma samples were collected at enrolment. SARS-CoV-2 viral load was measured in NPS by quantitative RT-PCR. Variant typing was conducted using the Allplex™ SARS-Cov-2 Variants I and II assays (Seegene Inc.). Anti-Spike IgG titres were measured by Anti-SARS-CoV-2 QuantiVac ELISA (Euroimmun). Mann-Whitney and Kruskal-Wallis tests were used for statistical analyses.

Results

Eighty-seven out of the 177 (49.2%) enrolled patients were vaccinated against SARS-CoV-2 and had a breakthrough infection, 77 of which (88.5%) received more than one dose (median 88 days after last dose, IQR 48-170). Ten vaccinees (11.5%) tested negative for anti-Spike IgG (median 86 days after the last dose, IQR 26-165). Vaccinees did not shown significant differences in the NPS viral load during the early infection phase when compared to unvaccinated individuals (90/177, control group) (p=0.4565), regardless of the infecting variant (Alpha: p=0.5787; Delta: p=0.4259; Omicron: p=0.4715). The majority of vaccinees with quantifiable IgG antibodies exhibited a higher viral load compared with IgG positive unvaccinated individuals (18/90, previously infected; p=0.0197), a difference mainly supported considering the subgroup of patients affected by the Omicron variant (38 vaccinees vs. 6

unvaccinated, p=0.0430). These differences were not observed among IgG-negative patients (vaccinated vs. unvaccinated, p=0.7855). Notably, vaccinees reported a median clinical recovery time of 11 days (IQR 9-15), while unvaccinated recovered in 15 days (IQR 11-22) (p<0.0001).

Discussion and Conclusions

These data support the evidence of SARS-CoV-2's ability to evade the immune system, demonstrating its capability to infect both vaccinated individuals and those previously infected, regardless of the circulating variant. The difference in viral load in IgG positive individuals probably depends on the specific infecting variant. Vaccinated patients exhibit a shorter clinical recovery time supporting the importance of SARS-CoV-2 vaccination to prevent severe COVID-19 in high-risk patients.

260 - MOUSE MODEL FOR ERADICATION OF STREPTOCOCCUS PYOGENES FROM THE UPPER RESPIRATORY TRACT

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Mouse model for eradication of Streptococcus pyogenes from the upper respiratory tract

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Introduction. Asymptomatic pharyngeal carriage plays a crucial role in the natural history of infections by Streptococcus pyogenes (Group A streptococcus, GAS), a strictly human pathogen with no other known reservoir. In the carrier state, S. pyogenes is typically associated to the lymphoid tissue of the tonsils and the epithelium of the upper respiratory tract. When mice are infected experimentally by intranasal route with S. pyogenes, bacteria readily home to the Nasal Associated Lymphoid Tissue (NALT), an organized mucosal-associated lymphoid tissue, functionally equivalent to the human tonsils. The murine NALT is located at the base of the nasopharyngeal cavity, in the nasal passages above the hard palate. It can be collected and subject to microbiological and immunological analyses.

In this work we tested the possibility to use the S. pyogenes infection of the murine NALT as a model to study the eradication of S. pyogenes from the upper respiratory tract. To obtain proof of concept that a microbicide delivered locally at the mucosal level can efficiently eradicate S. pyogenes carriage, we used purified PlyC, a phage lysin known to be bactericidal for S. pyogenes.

Materials and Methods. Outbred CD-1 mice were intranasally (IN) infected with 10^7 CFU/mouse of S. pyogenes 90-226. The weight loss of animals and the clinical score based on their healthy status was monitored. The NALT and nasal washes of infected mice were collected at different time points at the sacrifice of animals, and the kinetic of bacterial dissemination was investigated.

Results. S. pyogenes infection by induced weight loss in animals. The percent of infected animals peaked 4 days after the infection (90%) and was still high at day 5 (76%). When animals were treated with a single dose of PlyC, eradication of S. pyogenes from the murine upper respiratory tract was readily obtained.

Discussion and Conclusions. Experimental evidence was obtained that a local microbicide, the phage lytic enzyme PlyC, can eradicate S. pyogenes carriage from the NALT and the mucosa of the upper respiratory tract of experimental animals, in a mouse model of pharyngeal colonization.

[Artificial Intelligence was not used to produce this text]

263 - T-CELL IMMUNE DYSFUNCTION AND PRO-INFLAMMATION MARKERS IN COVID-19 PATIENTS PERSIST IN LONG COVID INDIVIDUALS DEFINING SPECIFIC PROFILES ASSOCIATED TO CARDIO-VASCULAR AND PSYCHIATRIC SYMPTOMS

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T-cell immune dysfunction and pro-inflammation markers in COVID-19 patients persist in Long COVID individuals defining specific profiles associated to cardio-vascular and psychiatric symptoms

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Introduction: Today, in the world are estimated 65 million individuals affected by Long COVID (LC) constituting a significant problem for public health. The complex alterations of the immune system and the immune-mediated multi-organ injury plays a key role in host response to SARS-CoV-2 infection and in the pathogenesis of COVID-19. The immune dysfunction has been also associated with the post-acute sequelae that characterize Long COVID. The aim of this work was to characterize T-cell immune dysfunction in COVID-19 patients and its persistent dysregulation in LC individuals.

Materials and Methods: Blood samples from 66 Acute COVID-19 patients (COV), 43 Healthy Donors (HD), and from 48 LC individuals (range: 7-48 weeks post-infection) were collected at Policlinic of Tor Vergata in Rome. The T cell differentiation markers were analyzed by flow cytometry and plasma levels of IL6, IL10, IL-8, TNF-alpha, and endothelin were evaluated using the fully automated Ella immunoassay platform. To identify associations between biomarkers in a multivariate manner, a factor analysis followed by varimax rotation and Kaiser normalization was performed (Principal Component Analysis, PCA).

Results: By means of flow cytometry analysis, a dysregulation of the markers of T-cell differentiation and exhaustion in COV patients with respect to HDs was found. Interestingly, a restore in the percentage of lymphocytes was observed in LC individuals similar to HDs levels but a chronically altered immune response associated with the differentiation and exhaustion processes in T CD8+ and CD4+ cells was observed, similarly to COV patients. Moreover, a persistent upregulation of circulating pro-inflammatory cytokines IL-6, TNF-alpha and IL-8, the regulatory cytokine IL-10, and endothelin 1

involved in vascular diseases was observed in COV and LC individuals. The Principal Component Analysis individuated specific multi-parametric profiles with immune dysfunction, biochemical and cytokines as markers that characterized LC with respect to the severity of acute Infection and to some specific cardio-vascular and psychiatric symptoms of LC. Discussion and conclusion: The obtained results offer new insights into characterization and possible intervention for those patients with long-lasting inflammatory and affecting post-infectious symptoms, some of which appear with delayed onset.

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266 - DIFFERENT ANTIGEN-SPECIFIC CD4+ AND CD8+ T-CELLS RESPONSE AGAINST HCMV PROTEINS IN PREGNANT WOMEN WITH PRIMARY INFECTION AND IN CONTROL SUBJECTS WITH REMOTE INFECTION

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Different antigen-specific CD4+ and CD8+ T-cells response against HCMV proteins in pregnant women with primary infection and in control subjects with remote infection.

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Introduction: Human cytomegalovirus (HCMV) is the most common cause of congenital infection and in 20% of the cases it results in long-term sequelae. The HCMV-specific T-cell response in primary infection may help define correlates of immune protection in pregnancy. In this study, the antigen-specific T-cell response against different HCMV proteins (IE-1, pp65, gB, gHgLpUL128L) was investigated in pregnant women with primary infection and in control subjects with remote infection to identify possible components of a protective vaccine.

Materials and methods: Thirty-five pregnant women with HCMV primary infection were enrolled within three months after the onset of infection and followed up until 24 months post infection. In addition, 30 HCMV-seropositive healthy adult subjects were enrolled as controls. The antigen-specific T-cell response was measured using cytokine flow cytometry after stimulation with IE-1, pp65, gB and gHgLpUL128L peptides pool.

Results: The gB-specific CD4+ and CD8+ T-cell response was higher in pregnant women with HCMV primary infection at 2 months after onset infection, while the subjects with remote infection showed a higher pp65-specific CD4+ T-cell response and a higher IE-1 and pp65-specific CD8+ T-cells response. Instead, gHgLpUL128L-specific CD4+ and CD8+ T-cells response was low in all groups of subjects. In addition, no difference was observed for the HCMV-specific CD4+ and CD8+ T-cell response between non-transmitting and transmitting pregnant women.

Discussion and conclusion: The T-cell response was higher against gB and pp65 proteins, at the beginning of infection and in seropositive subjects, respectively, therefore these proteins should be taken into consideration as candidates for a vaccine.

277 - LONG-TERM IMMUNE RESPONSE TO MRNA SARS-COV-2 VACCINES IN SOLID ORGAN TRANSPLANT RECIPIENTS

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Long-term immune response to mRNA SARS-CoV-2 vaccines in solid organ transplant recipients

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Introduction. Since the start of the vaccination campaign against SARS-CoV-2, most studies conducted on solid organ transplant (SOT) recipients have focused on characterizing the antigen-specific humoral response, while little is known about the spike-specific memory B cells (MBCs) response. Here, we characterized the phenotypes and the frequencies of receptor binding domain (RBD)-specific MBCs, beyond the humoral response, up to 2 years post-vaccination in these fragile subjects.

Materials and Methods. A cohort of 117 SOT, along with 54 healthy subjects, vaccinated against SARS-CoV-2 with mRNA-based vaccines, were enrolled in the clinical study. Blood samples were collected after the booster dose and 2 years following the first vaccination cycle to monitor the induction and the persistence of the immune response. Multiparametric flow cytometry was used to identify and characterize the phenotypes of RBD-specific MBCs induced by vaccination in naïve and infected-subjects. Wild-type and omicron BA.2 RBD were used as fluorescent probes to identify vaccine-specific B cells and clones cross reactive against BA.2 variant. The spike-specific antibody response was characterized by ELISA, and by a surrogate virus neutralization test, utilizing both wild-type and BA.2 Omicron variant antigens.

Results. The humoral response induced by SARS-CoV-2 vaccines in SOT recipients was significantly lower compared to healthy controls also after the third booster dose, both in terms of IgG levels and neutralizing capacity. Wild-type and BA.2 RBD-specific MBCs were still detectable in 94% and 93,2 % of SOT at 2 years after vaccination, respectively. Differences in phenotype were observed compared to controls, with a lower frequency of resting memory B cells. Clones specifically recognizing the BA.2 RBD variant were mostly IgM+, while clones specific for the wild type RBD or cross reactive with both, were IgG+ switched. Subjects low responder upon each vaccine administration were observed.

Discussion and Conclusions. Our findings show the induction and persistence of RBD-specific B cells in SOT recipients, even though significantly lower compared to controls, and a lower antibody response, possibly due to immunosuppressive treatments. The persistence of low-responders subjects despite multiple vaccine administrations, suggest the need of alternative approaches for these subjects. These findings could have a clinical relevance for planning vaccination schedules tailored for solid organs transplanted patients.

T07 RESISTENZA AI FARMACI E RISVOLTI TERAPEUTICI

7 - NEW INSIGHTS IN THE MECHANISM OF ACTION OF VOMG, AN ANTI-MYCOBACTERIUM ABSCESSUS DRUG CANDIDATE

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New insights in the mechanism of action of VOMG, an anti-Mycobacterium abscessus drug candidate

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Introduction

Mycobacterium abscessus (Mab) is an emerging opportunistic pathogen, of paramount concern for individuals with cystic fibrosis (CF). As the drug pipeline is limited, VOMG stands out as a new drug candidate with bactericidal activity against Mab (MIC=0.25 □g/ml), including multi-drug resistant clinical isolates, biofilm and other nontuberculous mycobacteria. VOMG has been shown to be active against a range of other CF-associated pathogens, including Staphylococcus aureus, Acinetobacter baumannii and fungi. Structure-activity relationship and drug-like ADME properties were established. The physicochemical properties of VOMG make it suitable for drug delivery by aerosol inhalation. Most importantly, VOMG activity in Mab-infected mice is comparable to that of amikacin, an aminoglycoside used in therapy. Transcriptomic analysis revealed the inhibition of essential metabolic pathways, upon VOMG treatment, particularly the downregulation of genes encoding the proteins involved in divisome and cell division (CD): FtsZ, SepF, EnvC, SteA, SteB, FtsQ. FtsZ is an essential protein that forms the scaffold of the divisome, and VOMG inhibits its activity by interfering with polymer stability. To in depth investigate the mechanism of action (MoA) of VOMG, we will construct conditional knock-down (KD) mutants in CD selected genes, to assess whether VOMG affects the activity of other CD proteins, employing microbiological and biochemical approaches. VOMG activity on FtsZ polymers will be investigated by cryo-electron microscopy.

Materials and Methods

KD mutants of the selected CD components are being constructed using the CRISPRi system. CD recombinant proteins are expressed in E. coli and purified. The interactions between VOMG and Mab proteins will be investigated by thermal shift assays, then, the VOMG effects on their activity will be studied by specific assays. FtsZ polymers will be visualized using cryo-electron microscopy.

Results

The construction of Mab KD mutants is progress.

Discussion and Conclusions

VOMG was patented under the co-ownership of UNIPV and Fondazione Italiana Ricerca Fibrosi Cistica (FFC). Our findings will pave the way for the development of new potential treatments against Mab infections, in particular for CF individuals.

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18 - MULTI-DRUG RESISTANT ENTEROBACTER CLOACAE ST145 CAUSING BLOODSTREAM INFECTION IN A HOSPITALIZED PATIENT: MOLECULAR CHARACTERIZATION OF RESISTOME AND VIRULOME

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Multi-drug resistant Enterobacter cloacae ST145 causing bloodstream infection in a hospitalized patient: molecular characterization of resistome and virulome

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Introduction. Enterobacter cloacae complex includes facultative anaerobic Gram-negative bacteria which are responsible for numerous nosocomial infections. The presence, in these Enterobacterales, of inducible AmpC-type beta-lactamases, confers them intrinsic resistance to penicillins, first- and second generation cephalosporins and their inhibitor combinations.

Materials and Methods. In the period from December 2023 to January 2024, E. cloacae strain was isolated from blood culture of a single patient affected by colorectal cancer. Blood cultures were processed using the BACTEC 9240 automated culturing system. The VITEK 2 system were applied for isolate identification, and the VITEK 2 GN09 was used to test the antimicrobial susceptibilities of all isolates. The whole-genome sequencing (WGS) was performed using the Illumina Miseq platform with a 2 x 300 paired-end run. Multilocus sequence typing (MLST) was performed using a BLAST-based approach. The PlasmidFinder 2.1 program was used to detect the incompatibility groups of plasmids.

Results. Sequencing of genomic DNA of E. cloacae gave knowledge of sequence typing (ST), mobile genetic elements, antibiotic resistance genes and virulence factors. The MLST analysis indicated the lineage ST145. The resistome of E. cloacae included genes which conferred resistance to aminoglycosides (aac(6')-Ib3, aph(3)-IIa, aac(6')-Ib-cr), beta-lactams (blaCTX-M-15, blaNDM-1, blaOXA-1 and blaACT-90) fosfomicin (fosA), chloramphenicol (catB3) and trimethoprim (dfrA14). The aac(6')-Ib-cr gene is a bi-functional gene which confer resistance to aminoglycosides and fluoroquinolones, in particular ciprofloxacin. The circulation of ARGs is facilitate by the presence of several insertion sequence such as IS6 family (IS6100, IS26), IS3 family (ISEhe3, ISEc52), IS1 family (ISKpn14), IS5 family (IS903), IS110 family (ISKpn43), IS1380 family (ISEc9), Tn5403 transposon and

IncFIB, IncFII and IncFIA plasmids. The molecular analysis allowed to identify in E. cloacae some virulence factors including terC, nlpl and clpk1 genes.

Discussion and Conclusions. In the present study we have characterized E. cloacae ST145 clinical isolates that harbors beta-lactamases belonging to molecular classes A, B, C and D. The plasmid-mediated blaACT-type genes are common in E. cloacae complex. The ACT-90 beta-lactamase is a ACT-14 variant with six amino acid substitutions. The production of carbapenem-hydrolyzing β-lactamase E. cloacae could render ineffective most beta-lactams.

41 - DISAPPEARANCE OF MACROLIDES RESISTANCE IN STREPTOCOCCUS PYOGENES INFECTIONS

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Disappearance of macrolides resistance in Streptococcus pyogenes infections

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Introduction

Several studies have indicated a global increase in the prevalence of invasive Streptococcus pyogenes infection (SPi) in younger and immunocompromised patients. Erythromycin has been the most commonly used treatment for SPi among to macrolide antibiotic. Across geographic regions, there are large variations in macrolide resistance rates, with low resistance in America, higher in Europe and highest in Asia where multicenter studies report resistance between 80 and 95%. The erythromycin-resistant strains in Campania region has increased exponentially and reached 54.6% in 2019. This study, in opposite with regional trend, showed macrolide-sensitive strains prevalence. To our knowledge, these are the first reported cases of disappearance of macrolide resistance in Streptococcus pyogenes.

Material and Methods

Streptococcus pyogenes were isolated from December 2023 to February 2024 at AORN Sant'Anna and San Sebastiano, Caserta, Southern Italy hospital. Clinical isolates were identified from different specimens, 44.5% from blood culture, 22, 2% from cerebral fluid (CSF), 11, 1% from pleural fluid, 11,1% from pericardial fluid, and 11,1% from drain. Isolates identification was performed by MALDI-TOF MS, and susceptibility antimicrobial agents were tested using VITEK-2 systems according to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2024). An analysis was made of the demographic, symptomatology, microbiology and treatment.

Result

The cases-patients were 60% female with a median age of 48 years. The most frequent clinical syndroms were fever, chills, hypotension, subcutaneus abscess and organ failure (kidney, liver, blood). On admission, liver and renal function tests were altered compared with normal values, as were D-dimer, C-reactive protein and lactate dehydrogenase (LDH), with a median range of 580, 23.71 and 895, respectively. All isolates were tested for susceptibility to macrolides and penicillin, and PCR was used to detect erythromycin resistance genes. The majority of isolates were susceptible to

clindamycin and only 11.1% of S. pyogenes isolates were resistant to erythromycin, clarithromycin, azithromycin and tetracycline. None of the erythromycin-resistant isolates showed costitutive Macrolides-Licosamide- Streptogamine β resistance (cMLSB).

Discussion and Conclusion

Macrolide resistance is decreasing in S. pyogenes strains, even in countries that have not reduced their use of macrolides, although the trend is not universal. This suggests that other yet unidentified forces may play a key role in determining the overall macrolide resistance rate. The analyzed Streptococcus pyogenes outbreak is important to improve infection control measures and antimicrobial stewardship program. An epidemiological study combined with whole genome sequencing could be crucial to define resistance limited to a number of genetic lineages in relation to other study population.

44 - THE IN VITRO GENETIC BARRIER TO RESISTANCE OF LENACAPAVIR IS NOT AFFECTED BY VIRAL SUBTYPE OR HEAVY TREATMENT EXPOSURE

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The in vitro genetic barrier to resistance of lenacapavir is not affected by viral subtype or heavy treatment exposure

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Introduction

Lenacapavir (LEN) is a HIV-1 capsid inhibitor currently approved for the use in heavily-treatment experienced (HTE) people with HIV (PWH). LEN resistance mutations associated were identified at positions 56, 66, 67, 70, 74, 105 and 107 of the p24 coding region. This in vitro study aimed to evaluate LEN susceptibility and genetic barrier in B and non-B subtypes derived from therapy naïve (TN) and HTE PWH.

Materials and Methods

Twenty-six NL4-3 recombinant viruses harbouring clinically derived GAG-PR region were generated from plasma samples collected from TN (n=15) and HTE PWH enrolled in the Italian PRESTIGIO registry (n=11). LEN half maximal inhibiting concentration (IC50) was measured through a TZM-bl cell line-based assay and fold-change (FC) susceptibility values were calculated with respect to the IC50 value of the NL4-3 wild type strain. In vitro resistance selection (IVRS) experiments were performed by exposing MT-2 cells infected with recombinant viruses and the NL4-3 control to increasing concentrations of LEN. Cultures were stopped when viral breakthrough was observed at approximately 100X LEN IC50 (2.56 nM) or after 105 days from the start of the IVRS. Sanger sequencing of the p24 coding region was performed at each viral breakthrough.

Results

None of the viruses harboured known LEN resistance mutations. Baseline susceptibility to LEN was comparable between HTE vs. TN (median FC 0.9 [IQR 0.3-1.6] vs. 1.6 [0.6-3.0], p=0.253, Mann-Whitney test) and between B (n=12) vs. non-B (n=14; 3 CRF02_AG, 3 F1, 1 each A1, C, D, G, CRF01_AE, CRF06_cpx, CRF40_BF, URF D/B) subtypes (median FC 0.7 [0.2-2.2] vs. 1.6 [0.7-2.5], p=0.141). By Kaplan-Meier survival analysis, the time to viral breakthrough was comparable among both B vs. non-B subtypes and HTE vs. TN PWH at approximately 10X (p=0.112 and p=0.551, respectively, log-rank test) and 100X LEN IC50 (p=0.226 and p=0.382, respectively). Known LEN resistance mutations emerged in 25/27 cultures including N74D (n=7), Q67H/R/K+N74D (n=6), Q67H/K+T107N/S (n=6), , N74D+T107N/D (n=3), Q67H+K70R+T107N (n=2), Q67H+K70R (n=2), and Q67H (n=1), while in two cases no emergent mutations were identified when the cultures were stopped. In addition, the non-polymorphic aminoacid substitutions F169L (with Q67H+T107TN), V86M (with Q67H+K70R) and E213D (with Q67H+T107N) were detected in three distinct cases each. The patterns of emerging mutations were equally distributed among B and non-B subtypes.

Discussion and Conclusions

In this study, the genetic barrier to resistance to LEN was not affected by viral subtype, previous failures to other ARV classes or long-time exposure to antiretroviral therapy. The frequent detection of emerging mutations in IVRS experiments indicates a low genetic barrier to resistance.

46 - COMPARATIVE ANALYSIS OF ISLATRAVIR AND TENOFOVIR IN VITRO ACTIVITY IN NRTI RESISTANT HIV-1 HARBORING THE M184V/I MUTATION

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Comparative analysis of islatravir and tenofovir in vitro activity in NRTI resistant HIV-1 harboring the M184V/I mutation

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Introduction

The HIV-1 RT M184V/I resistance mutation significantly reduces the activity of the NRTI lamivudine and emtricitabine, while slightly increases tenofovir susceptibility. In vitro studies have shown that M184V/I with other NRTI resistance mutations negatively affects susceptibility to the first-in-class nucleoside RT translocation inhibitor islatravir (ISL). This study aimed to compare the in vitro susceptibility to ISL, currently under clinical evaluation for the treatment of multidrug resistant HIV-1, and tenofovir alafenamide (TAF) in NRTI resistant isolates harboring the M184V/I mutation.

Materials and Methods

Recombinant viruses with clinically derived PR-RT were generated from 23 samples collected from heavily treatment experienced (HTE) people living with HIV (PLWH) enrolled in the Italian PRESTIGIO Registry having multiple NRTI mutations, including M184V/I, except for one case with M184V only. All but three viruses included also NNRTI mutations. In vitro susceptibility to both drugs was determined through a TZM-bl-based assay and fold-change (FC) values were calculated with respect to the IC50

value obtained with the wild-type NL4-3 strain. Genotypic and phenotypic susceptibility to TAF were compared using the HIVdb Stanford algorithm v9.5.1 and the Monogram phenotypic clinical FC cut-off values (1.4-4.0).

Results

The median FC values of ISL and TAF were 7.3 [IQR 3.9-13.7] and 2.7 [IQR 1.3-4.5], respectively ($p<0.0001$, Wilcoxon signed rank test). Although neither the number of TAMs nor that of NRTI mutations correlated with ISL FC, the mutational pattern M184I + 9 NRTI mutations was associated with the highest ISL FC (37.8), while the virus collected from the same PLWH at a different time point without K70Q showed a lower FC (5.4). Interestingly, the presence of the NNRTI mutation V106I was associated with the third largest ISL FC (22.6), confirming a possible cooperative effect between M184V and V106I in reducing susceptibility to ISL. The presence of K65R was associated with two among the highest FC values for TAF (7.7 and 4.9). When excluding samples with K65R, TAF FC values positively correlated with the number of NRTI RAMs and TAMs ($p=0.012$ and $p=0.0004$, respectively, Spearman's rank test). Genotypic susceptibility to TAF was in fair agreement with phenotypic susceptibility, however there were both cases of over- and under-estimation of genotypic resistance.

Discussion and Conclusions

HTE PLWH harbouring the M184V/I virus might benefit more from TAF than ISL, however the association between ISL FC and in vivo effectiveness remains to be defined. Since TAF and ISL are both likely to be the candidate NRTI in HTE PLWH, phenotypic testing may contribute to define their role in the context of complex mutational patterns.

51 - PHAGE DISPLAY-DERIVED MONOCLONAL ANTIBODIES AS POSSIBLE VACCINES AGAINST THE STAPHYLOCOCCAL CELL-WALL ANCHORED COLLAGEN ADHESIN CNA

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Phage display-derived monoclonal antibodies as possible vaccines against the staphylococcal cell-wall anchored collagen adhesin CNA

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Introduction: Staphylococcus aureus is a human commensal bacterium; however, its pathogenic form is responsible for infections including endocarditis, osteomyelitis, and bacteremia. Since the phenomenon of antibiotic resistance has been estimated to cause 10 million per year of deaths by 2050, the aim of this study was to select human monoclonal antibodies (mAbs) through antibody-phage display as potential vaccines against staphylococcal infections to use in clinics. Materials and Methods: The mAbs were in vitro selected through antibody-phage display against the collagen-binding adhesin (CNA) expressed by S. aureus and they were biochemical, biophysical and biological characterized. 18 unique mAbs were selected and characterized with Enzyme Linked Immunosorbent Assay (ELISA) and Surface Plasmon Resonance (SPR) techniques to determine the binding and the affinity for the antigen. The activity was evaluated on recombinant purified adhesins and on adhesin-expressing bacteria. Finally, the epitopes of the most interesting mAbs were experimental and in silico mapped. Results: The results on the antibody-antigen binding demonstrated that they not only bind to CNA, but one can also recognize an adhesin expressed from another Gram-positive bacterium. We discovered that 2 mAbs were able to neutralize the in vitro infection (either by inhibition or displacement) of adhesins-expressing bacteria. Conclusions: In conclusion, completely human mAbs have been selected for the first time against the staphylococcal CNA and 2 antibodies showed an interesting neutralization activity against Staphylococcus aureus and Enterococcus faecium bacteria. A 3D model with eukaryotic cells will be introduced to study the mAbs activity before animal models studies for assessing the potential of mAbs as therapeutic agents for human applications.

54 - PROJECT OVERVIEW: COMPLEX INTERACTION BETWEEN THE HUMAN PROTEOME AND THE HIV GENOME

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Project Overview: Complex interaction between the human proteome and the HIV genome

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Introduction. The complex nature of HIV presents challenges in finding a cure despite advances in treatment. Focus has shifted to host interactomes influencing infection outcomes due to limited druggable HIV proteins. Understanding the interplay between the human proteome and HIV genome is crucial for identifying therapeutic targets. Techniques like LDP and VIR-CLASP elucidate interactions between integrated proviral DNA or incoming virion RNA and cellular proteins, shedding light on early infection mechanisms. These approaches offer insights into addressing treatment efficacy, drug toxicity, and drug resistance. Materials and Methods. The VIR-CLASP technique involves culturing HIV-1 strains in MT-4 cells with 4-thiouridine (4SU) to label viral genomes, aiding RNA-protein cross-linking. Ultracentrifugation with a sucrose cushion purifies the virus, removing free 4SU for specificity. UV light exposure induces cross-linking in infected cells. Solid-phase reversible immobilization (SPRI) optimizes buffer conditions for selective isolation of cross-linked RNA-protein complexes, recovering proteins bound to 4SU-containing RNA viral genomes. Eluted complexes undergo RNA digestion with nuclease, and protein identification is achieved through Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The LDP technique employs U1 cells with integrated HIV genomes in the DNA phase. This method utilizes a recombinant primer for genome amplification via PCR, followed by a secondary amplification using a biotinylated primer. Subsequently, 500 bp of biotinylated dsDNA will be incubated with nuclear and cytoplasmic protein extracts from the cell line. After trypsin digestion, proteins interacting with DNA will be identified by LC-MS/MS. Results. VIR-CLASP demonstrates versatility in its applicability to diverse RNA viruses and offers rapid implementation due to its utilization of SPRI paramagnetic bead-based technology. Unlike methods requiring sequence-specific optimization for viral RNA purification, VIR-CLASP streamlines comparative analyses across various

time points, cell lines, and viral strains. By enhancing our comprehension of early viral replication events. The novel LDP method makes traditional pull-down assays more effective and efficient for identifying and studying long regions of proteins that interact with DNA. VIR-CLASP and LDP pave the way for new therapeutic and prophylactic interventions in HIV infection. Discussion and Conclusions. A thorough comprehension of host-virus interactions offers potential for innovative antiretroviral strategies that can complement current therapies and tackle issues related to drug resistance and treatment ineffectiveness.

57 - CLOSTRIDIOIDES DIFFICILE ANTIBIOTIC SUSCEPTIBILITY OVER THE YEARS: EVALUATION OF RESISTANCE PROFILE IN CLINICAL ISOLATES AT PADUA UNIVERSITY HOSPITAL

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Clostridioides difficile antibiotic susceptibility over the years: evaluation of resistance profile in clinical isolates at Padua University Hospital

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Introduction

Clostridioides difficile is responsible for opportunistic infections in patients treated with broad-spectrum antibiotics. The annual incidence of C. difficile associated diarrhea (CDAD), affecting hospitalized and immunocompromised patients, has showed a worldwide significant increase in recent decades. Since pathogens' antibiotic resistance is rapidly changing under the therapies' selective pressure, we assessed whether the antibiotic susceptibility profile of C. difficile strains circulating at Padua University Hospital has modified over a 14-years period.

Materials and methods

C. difficile strains were isolated from fresh stool samples of patients with CDAD between 2010 and 2018 (before fidaxomicin introduction in Padua Hospital) and between January 2023 and March 2024 at the Microbiology Unit of Padua University Hospital. Isolates were identified by MALDI-TOF analysis (99.9% confidence). Susceptibility to vancomycin, clindamycin, moxifloxacin, metronidazole, ertapenem, imipenem, meropenem, piperacillin/tazobactam, amoxicillin/clavulanic acid, ampicillin and penicillin was determined by broth microdilution method with a commercial kit (MICRONAUTS-S Anaerobes MIC, Bruker). Resistances were confirmed with the E-test method. Moreover, fidaxomicin sensitivity was tested by agar diffusion method (20 micrograms/disk). CLSI and EUCAST Breakpoint were used to define susceptibility profile.

Results

Thirty C. difficile strains from 2010-2018 and 37 from 2023-2024 were analyzed. All isolates resulted susceptible to amoxicillin/clavulanic acid, ertapenem, meropenem, piperacillin/tazobactam, metronidazole and vancomycin. While the resistance rate to moxifloxacin and clindamycin significantly decreased ($p<0.01$), the incidence of resistance to ampicillin significantly increased in

strains isolated in 2023-2024 compared to 2010-2018 strains ($p<0.05$). Contrary to, incidence of resistance to imipenem and penicillin was unaffected. The inhibition zones of fidaxomicin ranged between 15 mm and 30 mm in the 2010-2018 strains, whereas in the 2023-2024 group the inhibition zones varied between 15 and 35 mm (20.48±0.5 versus 20.60±0.5, respectively, p= n.s.).

Discussion and Conclusions

This study shows a modification in the sensitivity/resistance profile of C. difficile clinical isolates at Padua University Hospital. Although all strains retain sensitivity to metronidazole and vancomycin, a significant increase of ampicillin resistance was revealed. Although a wide range of isolates' sensitivity to Fidaxomicin was revealed, no changes in overall sensitivity to fidaxomicin was detected following introduction of this antibiotic in clinical practice. These data support the need of continuous antibiotic susceptibility monitoring for this nosocomial-acquired pathogen.

93 - PHENOTYPIC AND MOLECULAR SURVEY OF PSEUDOMONAS AERUGINOSA ISOLATES IDENTIFIED IN A TEACHING HOSPITAL, SOUTHERN ITALY

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Phenotypic and molecular survey of Pseudomonas aeruginosa isolates identified in a Teaching Hospital, Southern Italy.

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Introduction: Extensively drug-resistant (XDR) and multidrug-resistant (MDR) of Pseudomonas aeruginosa isolates were associated to treatment failure and increased mortality. Recently, carbapenem-resistant P. aeruginosa (CRPA) strains are an increasingly important problem globally. Herein, we screened the antimicrobial resistance profile of CRPA clinical isolates by classical and molecular assays. Materials and Methods: Between December 2019 and March 2024, 18 CRPA isolates were collected in the Dulbecco University Hospital, Catanzaro. Antibiotic susceptibility testing was performed by broth microdilution and Vitek®2 (bioMérieux) methods. Susceptibility to Cefiderocol (CFDC) was determined using Kirby-Bauer's disk diffusion technique, according to EUCAST guidelines. The bla-VIM and bla-NDM genes were sequenced by Sanger technology. Enzyme gene types using the Basic Local Alignment Search Tool nucleotide (BLASTn) algorithm were analysed. ProtCompB (Softberry software) was performed to predict sub-cellular localization of carbapenemases. Results: the CRPA strains were isolated from blood culture (n=3), urine (n= 4), bronchial aspirate (n=3), bronchoalveolar lavage (n= 2), throat swab (n= 4), rectal swab (n= 1) and ulcer swab (n=1). All isolates were also resistant to penicillins. CFDC non-susceptibility was observed in three out of 18 P. aeruginosa isolates. Eleven out of 18 strains carried VIM-70, 6/18 harbored VIM-1 and one isolate (#14) was VIM-gene negative. Additionally, another one isolate (#3) carrying both VIM-70 and NDM-64. Seventeen strains were bla-NDM negative. VIM and NDM sequences didn't display additional mutations compared to wild-type reference strains (NG_050336.1, NG_068039.1, PP238488.1). According to computational method, VIM enzymes and NDM-64 were predicted in extracellular location (9.7 and 4.4 integral score, respectively). Discussion and Conclusions: P. aeruginosa rapidly becomes CFDC resistant during antibiotic therapy. Additionally, meropenem and imipenem drugs, administered as empirical therapy, may be promoting selection of carbapenem-resistant strains. In this study, we characterized the two major metallo-beta-lactamases and phenotypic resistance to CFDC involved in the spread of Eskape pathogens. We detected one isolate (#3) carrying both VIM and NDM genes and three CFDC resistant isolates. Overall, all isolates carried

VIM secreted in the extracellular environment. In conclusion, this survey provides data about phenotypic and genotypic properties of P. aeruginosa emerged in our Hospital and may contribute in improving infection control measures and surveillance system.

99 - MICOCIDAL EFFICACY OF OZONIZED OILS IN DIFFERENT FORMULATION ON FOUR DIFFERENT CLADE OF CANDIDA AURIS RESISTANT TO CURRENT THERAPIES

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RESISTENZA AI FARMACI E RISVOLTI TERAPEUTICI

Micocidal efficacy of ozonized oils in different formulation on four different Clade of Candida auris resistant to current therapies

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Introduction

The World Health Organization has brought the issue of the increasing resistance of Candida pathogenic strains to antifungals due to their immoderate use in human infection and agriculture. The climatic change we are experiencing with the increase in temperatures and humidity has led to a greater proliferation of fungal species which have increased their virulence among plants, animals and humans. In this context Candida auris is one of the pathogens of highest interest, characterized by a remarkable tendency to multidrug resistance (MDR), resistance for a long time in the environment and high transmissibility. Recently, ozonated sunflower seed oil (OSO) has been demonstrated as a good mycocide, comparable or better than common antifungal drugs. In our studies we focused on the mycocide power of OSO, even conveyed by substances commonly used in pharmacological preparations on different known Clades of C. auris.

Materials and Methods

A commercial ozonized sunflower oil (OSO) was tested against a panel of four clinical isolates belonging to different C. auris strains (clade I, II, III and IV). Fungi were challenged with different oil concentrations and conveyance to assess antimicrobial resistance/susceptibility by agar diffusion and microdilution assays. The data were compared with reference antimycotic agents (fluconazole and amphotericin B). Cytotoxicity was also evaluated in epithelial cells.

Results

All C. auris was susceptible to the tested formulation, the drug delivery system tested keeps the antifungal properties unchanged suggesting its use to sustain and improve the repair of damaged tissue.

Discussion and Conclusions

Overall, these results suggest that OSO may be taken into consideration to complement or substitute the topic therapy against C. auris in particular when in presence of MDR strains.

C. auris is susceptible to the OSO in different formulations indicating that it does not express any intrinsic resistance mechanisms to these substances. This paves the way for the preparation of therapeutic formulations against this OSO-based pathogen.

100 - KILLING TWO BIRDS WITH ONE STONE: EMERGENCE OF COLISTIN AND CEFIDEROCOL RESISTANCE IN A MUCOID MDR ACINETOBACTER BAUMANNII UNDER COLISTIN PRESSURE

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Killing two birds with one stone: emergence of colistin and cefiderocol resistance in a mucoid MDR Acinetobacter baumannii under colistin pressure

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Introduction
Acinetobacter baumannii (Ab) is one of the major cause of health care-associated infections, characterized by elevated antibiotic resistance. For the treatment of infections caused by multidrug-resistant (MDR) Ab, colistin (COL) and cefiderocol (FDC) are considered last-resort antibiotics; however, resistance to these drugs is increasingly being reported.
Here we describe the rapid emergence of concomitant resistance to COL and FDC in a hypermucoid Ab (HM-Ab) strain isolated from a cystic fibrosis (CF) patient.
Materials and Methods

Four isolates of Ab were subjected to susceptibility test and Whole Genome Sequencing (WGS). An HM and a low mucoid (LM) phenotypes were isolated from patient sputum obtained during the delayed CF diagnosis made in our Centre. Other 2 Ab strains, both HM, were isolated 5 months later. During this time, the patient had received 15 days of intravenous meropenem and tobramycin, followed by continuous and alternating inhaled antibiotic therapy with tobramycin and COL for maintenance therapy against a Pseudomonas aeruginosa infection.

Results

Both HM-Ab and LM-Ab strains from the first sample had a MDR profile, with sensitivity to COL and low Minimum Inhibitory Concentration (MIC) to FDC. In the second sample, LM-Ab was no longer detectable, while HM-Ab had 2 different susceptibility profiles to COL and FDC.
All 4 Ab isolates belonged to the ST2 clone and possessed a plasmid carrying the carbapenemase OXA-23. HM-Ab had missense mutations in the wzc (ptk) gene of capsular locus, namely A531V and M653V in the first sample and M653V in the second one. The HM-Ab strain with high MICs to COL and FDC in the second sample had missense mutations in pmrB and piuA genes, respectively.

Discussion and Conclusions

Infections caused by MDR Ab are difficult to treat, and resistance is emerging even to therapies long considered as last resort. In our case, LM-Ab was presumably eradicated by antibiotic therapies, while HM-Ab adapted to COL through LPS modification by altering PmrB, the in vivo predominant mechanism. Unfortunately, the COL resistance strain also had FDC resistance caused by a mutation in a TonB-dependent siderophore receptor. FDC resistance emergence during treatment, as well as cross-resistance induced by ceftazidime/avibactam and ceftolozane/tazobactam have already been described, whereas cross-resistance between COL and FDC was only deemed potential. Considering FDC heteroresistance prevalence (60%) in carbapenem-resistant Ab, we hypothesize that COL selected for a subpopulation of HM-Ab already carrying mutation conferring resistance to FDC.

Given the reduced treatment options for MDR Ab infected patients, this case emphasizes the need to carefully administer last resort antibiotics.

117 - EFFECT OF LAST-LINE ANTIBIOTICS ON MATURE BIOFILM OF KLEBSIELLA PNEUMONIAE CARBAPENEMASE-PRODUCING KLEBSIELLA PNEUMONIAE STRAINS

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Effect of last-line antibiotics on mature biofilm of Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae strains

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Introduction: Bloodstream infections caused by Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria are a growing problem that require an adequate choice of therapy especially in patients with devices like central venous catheter (CVC). In particular, Klebsiella pneumoniae KPC is able to cause life-threatening CVC-related infection due to the formation of biofilm on catheter surface. In this setting, possible therapies include removal of the device or the treatment with antibiotics capable of disrupting the biofilm to clear the infection and avoid the possibility of reinfection. In this study, we evaluated for the first time the effect of 3 last-line antibiotics on K. pneumoniae mature static biofilm model.

Materials and Methods: Klebsiella pneumoniae strains were isolated from patients diagnosed with device-related bloodstream infections at Perugia Hospital. Minimum inhibitory concentrations (MICs) of meropenem/vaborbactam, imipenem/relebactam and ceftazidime/avibactam were determined in vitro. Biofilm formation was evaluated using 96-well polystyrene microtiter plates and mature biofilms were treated with concentrations of antibiotics ranging from 1x to 16x the MICs previously determined. After treatments, biofilm biomass and metabolic activity were assessed by crystal violet and XTT reduction assay, respectively.

Results: Twelve selected strains were tested for their capacity to form biofilm in vitro. In our experimental conditions, 8 out of 12 strains showed to be strong biofilm producers after 48 h of incubation with medium renewal after 24 h. The treatment of strong biofilm producer strains with the selected antibiotics led to a correlated decrease in both biofilm biomass that metabolic activity, indicating the ability to significantly disaggregate pre-formed biofilms. However, K. pneumoniae biofilm showed different susceptibility towards antibiotics, with effects at clinically significant doses for most of the selected strains.

Discussion and Conclusions: Biofilm formation on medical device is a recurring threat in hospital settings and the ability of antibiotics to efficiently reduce it is of great importance. Our results showed that the reduction of biofilm in term of biomass and metabolic activity is often reached at

concentrations much higher than the MICs, indicating the need of high dose treatment regimens for prolonged time. Furthermore, the concentrations of antibiotics could exceed clinically achievable doses, making the treatment ineffective. To avoid therapy failure, the combination of different antibiotics could be helpful in reducing the effective dose required to affect biofilm formation or disaggregation. In the light of this results this study will involve the use of combined therapies on K. pneumoniae KPC biofilm.

121 - CHARACTERIZATION OF NEW CHALCONE DERIVATIVES AS PROMISING LEADS TO TREAT TUBERCULOSIS

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Characterization of new chalcone derivatives as promising leads to treat tuberculosis

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Introduction

The spread of strains of Mycobacterium tuberculosis (Mtb) resistant to currently antitubercular drugs is a serious global public health problem. In this context, the discovery of novel antitubercular agents is necessary and urgent. Moreover in chronic infectious diseases, such as tuberculosis (TB), exacerbated inflammation contributes to severe lung pathology, leading to tissue necrosis, cavities formation and the promotion of mycobacterial dissemination and transmission. In this context, new candidates exhibiting dual activity, antimycobacterial and anti-inflammatory, can provide an important therapeutic advantage in the aggressive forms of TB. Chalcones are essential intermediate compounds in flavonoid biosynthesis in plants, and present many biological activities, including anti-inflammatory and antimycobacterial. In this study a series of chalcones were synthesized to characterize them as antitubercular agents.

Methods

In silico screening was performed using PubChem, SEA, and ChemBL, followed by molecular docking (Autodock 4.2). Cytotoxicity and inhibitory effect on TNF-α and IL- β production were evaluated in RAW 264.7 macrophages. MIC90 was determined by Resazurin Microtiter Assay. The isolation of Mtb mutants will be performed by plating an exponential growth phase culture of H37Rv onto 7H10 medium containing different concentrations of the compounds. Genomic DNA of Mtb mutants will be collected for WGS analysis.

Results

In the present study, 41 chalcone derivatives were tested for their activity against Mtb H37Rv, immunomodulatory properties and cytotoxicity in RAW 264.7 macrophages. Six of them with the highest activity (MIC90: 10-30 μM), and selectivity (CC50 > 70 μM) were considered for further

investigation. Through an in silico screening approach, followed by molecular docking, Mtb protein tyrosine phosphatases (PtpA and PtpB) were identified as potential targets for these compounds. In literature, kinetic studies describe chalcones as reversible competitive inhibitors for these enzymes interacting with the amino acid residues Thr12, Arg17, Trp48, and His49 in the active site of PtpA. To validate these enzymes as targets also of these derivatives, resistant mutants will be selected in vitro growing Mtb on 7H10 containing the drug and re-tested individually for their MIC to reconfirm their resistance. To identify the polymorphism responsible for the observed resistance phenotype, whole genome sequencing (WGS) of the isolated resistant mutants will be performed.

Discussion and Conclusions

In conclusion, new antitubercular chalcones were identified and Mtb tyrosine phosphatases were predicted as molecular targets. These preliminary data encourage further studies for the characterization of these compounds as drug leads in TB cure.

131 - SHIFTING TO A DOUBLE BETA-LACTAM TREATMENT FOR GRANULICATELLA ADIACENS INFECTIVE ENDOCARDITIS? ANALYSIS OF GRANULICATELLA ADIACENS PENICILLIN-BINDING PROTEINS (PBPS) CEFTOBIPROLE INHIBITION

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Shifting to a double beta-lactam treatment for Granulicatella adiacens infective endocarditis? Analysis of Granulicatella adiacens Penicillin-Binding Proteins (PBPs) ceftobiprole inhibition

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Introduction: As in enterococcal infective endocarditis (IE), the international guidelines recommend treating Granulicatella adiacens IE with a combination of beta-lactam plus gentamicin. However, conscious of the nephrotoxicity risk caused by aminoglycoside, we evaluated the synergistic activity of double beta-lactam combinations and their affinity towards Penicillin-Binding Proteins (PBPs) via competition assays using a labelled penicillin (BocillinTM FL). Materials: The clinical isolate was identified through 16S RNA sequencing. Then, the antimicrobial susceptibility testing was performed by Gradient-test and the MICs evaluated using CLSI M45 breakpoints for Granulicatella spp.. To evaluate synergistic activity, we carried out Gradient-Cross (90° angle) method and interpreted the results by FIC index. The binding of β-lactams to PBPs was evaluated performing competition assay exposing cells to ceftobiprole (BPR) at different concentrations (1/2- , 1-, 2-, 4- fold MIC value) and then to BocillinTM FL, a fluorescent penicillin which compete with the antibiotic for the same catalytic site of PBPs. PBPs were detected using Typhoon FLA 9500 and the 50% inhibition concentration (IC50) was calculated. Results: The strain was susceptible to vancomycin and imipenem, resistant to ceftriaxone and cefotaxime and not-susceptible to penicillin and ampicillin. The better synergistic activity was registered testing double beta-lactam using ampicillin plus ceftriaxone or ceftobiprole at 1-fold their MIC values. By in silico analysis (AAT Bioquest, Inc.) of the G. adiacens ATCC 49175

genome (acc. N. NZ_CP102283) we found 4 High-Molecular-Mass (HMM) PBPs and 1 Low-Molecular-Mass (LMM) PBPs. Then, evaluating their mobility on SDS-PAGE, we observed that the HMM PBP of 77 kDa, designated as PBP2 in G. adiacens ATCC 49175 genome, was strongly inhibited at BPR MIC value. Besides, BPR activity toward further PBPs was confirmed showing affinity to another HMM PBP of 80 kDa, which belong to PBP1 family. Conclusions: The combination of ampicillin and ceftobiprole may determine a favourable synergy and enhanced killing activity due to the distinct and complementary saturation of diverse PBPs. These results underline the importance of using double beta-lactam treatment option for G. adiacens IE to reach a more promising outcome, in particular when it is preferable not to use aminoglycosides when they are not suitable or tolerated.

137 - A SNAPSHOT ON HIV-1 SUBTYPES AND DRUG RESISTANCE IN PEOPLE LIVING WITH HIV PRESENTING IN 2021-2023 AT THE UNIVERSITY HOSPITAL IN PALERMO, ITALY

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A snapshot on HIV-1 subtypes and drug resistance in people living with HIV presenting in 2021-2023 at the University Hospital in Palermo, Italy

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Introduction

HIV-1 infection remains a major public health concern despite increased use of HIV therapy. In fact, HIV is characterized by high rates of viral replication and mutations that can induce the emergence of drug resistance, affecting treatment efficacy and clinical outcomes. At present, the prevalence of pre-treatment drug resistance mutationsis unclear. This study investigated the burden of HIV resistance in both naïve to anti-retroviral treatment (ART) and experienced people living with HIV(PLHIV).

Materials and Methods

Genotyping and drug resistance testing was performed on 122 naïve to treatment and experienced PLHIV admitted from June 2021 to October 2023 to the Infectious and Tropical Diseases Unit of the AOUP "P.Giaccone" University Hospital in Palermo, Italy, by using the HIV-1 Solution V2 Kit (Arrow Diagnostics) with the MiSeq Illumina NGS platform. Routine viral load assessment was performed before genotyping by the dual-target COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (v.2.0, Roche Diagnostics S.p.A., Monza, Italy).

Results

The study population included 88 (72%) ART-naïve individuals and 34 (28%) individuals with virological failure; 74% were male and the median age was 46 years (IQR 37–53). The country of origin was generally Italy (79%), followed by Africa (17%), South America (2%), and Eastern Europe (2%). Among ART-naïve PLHIV, median HIV viral load and CD4 T cell count were 259,000 copies/ml (IQR 46, 125–897,000) and 184 cells/µL (IQR 51–390), respectively. The main HIV subtype was B (54%), but also A1,

C, F1, and G were reported (18%), as well as several Circulating Recombinant Forms (28%). Resistance-associated mutations were mostly observed to NNRTIs (10 cases, 5 ART-naïve), but also to NRTIs (6, all experienced to treatment), PIs (1 ART-naïve), and INIs (6, 3 ART-naïve). INIs resistance did not affect new generations drugs bictegravir and dolutegravir. However, reduced susceptibility to BIC and/or DTG was reported in 6 cases (3 ART-naïve). Resistance to cabotegravir was observed in 4 cases (1 ART-naïve) and reduced susceptibility in 3 (2 ART-naïve). A single case of simultaneous resistance to 2 classes of drugs was detected. The most common mutations associated with resistance were M184V and K65R for NRTIs, K103N/T for NNRTIs, L90M for PIs, and Q148K/H and S147G for INIs.

Conclusions

Our study highlighted an elevated rate of non-B HIV subtypes (46%) and HIV drug resistance (18%). Both experienced and naïve to treatment PLHIV presented resistance mutations, but NRTIs resistance was observed in experienced only. No resistance to new generation INIs was observed but cabotegravir. PIs class was associated with a low burden of resistance.

144 - ASSESSING THE MOLECULAR BASES OF FUSIDIC ACID RESISTANCE IN BORDERLINE OXACILLIN RESISTANT STAPHYLOCOCCUS AUREUS CLINICAL STRAINS

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Assessing the molecular bases of fusidic acid resistance in borderline oxacillin resistant Staphylococcus aureus clinical strains

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Introduction

The recent emergence of fusidic acid (FA)-resistant Staphylococcus aureus has underscored the importance of active surveillance in isolating these strains. Such strains can affect both methicillin-resistant and methicillin-susceptible S. aureus isolates. We recently isolated four FA-resistant strains and here we report their phenotypical and genotypical characterization.

Materials and Methods

Three out of four S. aureus clinical strains were obtained from two hospital units in Catania, while the fourth was from Palermo. In vitro antibiotic susceptibility testing was conducted using the E-test method. Whole Genome Sequencing was performed using the Illumina MiSeq Platform. Data analysis, including identification of Sequence Type, resistome, and virulome profiles, was carried out using QIAGEN CLC Genomics Workbench software. Further analysis involving the agr locus, SCCmec, spa-type, and plasmid analysis utilized bactopia, AgrVATE, staphopia-sccmec, spaTyper, and plasmidfinder software.

Results

Genotypic characterization revealed that the strains belonged to four distinct Sequence Types: ST630, ST8, ST15, and ST1. FA resistance was associated with mutations in the fusA gene in 2 out of 4 strains (with MIC values ranging from 8 to 16 mg/L), one strain with fusB (8 mg/L), and one with fusC (32 mg/L). Additionally, one case exhibited resistance to mupirocin (MUP), related to the presence of the

mupA gene (MIC value >1024 mg/L). While the strains were generally susceptible to main anti-Gram-positive agents, high MIC values (4 mg/mL) were observed for cefoxitin in 3 out of 4 cases, leading to their categorization as Borderline Oxacillin-Resistant Staphylococcus aureus (BORSA). Virulence gene content was complex and diversified, varied among the strains, with one testing positive for the pvl gene.

Discussion and Conclusion

As an antibacterial agent FA requires continuous evaluation to enhance treatment strategies, especially for its role in treating staphylococcal skin infections. Resistance to FA is articulated, involving point mutations in chromosomal genes (fusA or fusE) or association with mobile genetic elements such as Resistance Islands and Plasmids (fusB-fusC). Understanding FA resistance depends on studying mechanisms that involve the gene encoding the EF-G elongation factor and accessory/regulatory genes. Alongside with MUP, FA was demonstrated to be equally or more effective than oral treatment for skin and soft skin infection. Monitoring the resistance to these antibiotics may help to manage and to eradicates S. aureus MUP and FA resistant carriers as well as to contrast the spread of important virulence determinants.

155 - MANAGING RESISTANCE AND VIRULENCE: THE HIDDEN WORLD OF ENTEROCOCCUS FAECALIS FROM INFECTIVE ENDOCARDITIS

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Managing resistance and virulence: the hidden world of Enterococcus faecalis from infective endocarditis

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Introduction: Enterococcus faecalis is responsible for 10% of all cases of infective endocarditis (IE) and is characterized by challenging treatment and high-rate recurrence. We investigated the resistome, virulome and Penicillin-Binding-Protein (PBP) diversity of clinical isolates belonging to infective endocarditis (IE) of patients who received ampicillin dual beta-lactam therapy. Materials and Methods: We analyzed by whole-genome sequencing the genomic diversity of 10 contemporary E. faecalis isolates from patients affected by IE, treated with double beta-lactams. Biofilm production and bactericidal/synergistic activity of ceftobiprole plus ampicillin were also evaluated. Results: Genetically diverse E. faecalis were demonstrated, belonging to 9 different STs, among which ST40, ST268 and ST220 strictly correlated. The study of Penicillin-Binding-Proteins (PBP) gene mutations unveiled distinct PBP signatures, suggesting an evolutionary convergence in these isolates. Tolerance and/or persistence behaviors emerged from time-kill assays among those isolates with distinctive PBP signatures. ST 21 isolates showed an adenine insertion in pbp4 promoter region, associated with pbp4 overexpression that increased BPR MIC values, not affecting BPR bactericidal activity. Across all the genomes were identified six common antimicrobial resistance determinants (AMR) to macrolide (MLS)mph(D)], trimethoprim (dfrA), drug and biocide (efrA, efrB), multidrug efflux pump (emeA), clindamycin quinupristin-dalfopristin, dalfopristin [(MLS)lsaA)]; ant(6)-Ia, tet(M) and erm(B) genes were less frequent. Nine core virulence factors encoding biofilm formation (bopD), immunomodulation (cpsA, cpsB), adherence (ebpA, ebpB, ebpC, srtC), endocarditis antigen (efaA), surface fibrinogen-binding protein (fss1), were further identified. fsrABC-gelE-sprE agr-like genes, involved in biofilm formation, were almost always present. Only 2 strains showed prgB gene coding for an adhesin with a major a role in cellular aggregation and robust biofilm formation, one of this also

carrying the cyl-operon/asa1 (cytolysin and aggregation substance genes), carried by the pheromone-responsive, virulence plasmids pCF10 and pAD1, respectively. Two further strains showed several cps genes coding for capsular polysaccharide enzymes, with anti-phagocytosis/immune-evasion/immune-modulation properties. All but three isolates were ace defective, lacking an adhesin involved in host-cell attachment and immune-stimulation, suggesting a predisposition of these strains to evade the immune-system, persist and lead to IE relapse. The genomes also carried plasmids belonging to 5 replicon families related to resistance and virulence. Discussion and conclusions: This study provides valuable insights into the genomic characteristics of E. faecalis responsible for IE, including antimicrobial resistance, virulence factors, and penicillin-binding protein types. Understanding the genetic variations that influence phenotypic changes could enhance the ability of E. faecalis to survive in the presence of antibiotics and immune responses, potentially leading to recurrent infections.

156 - VANCOMYCIN VARIABLE ENTEROCOCCI (VVE) LINKED TO SEVERE BLOODSTREAM INFECTIONS IN IMMUNOCOMPROMISED PATIENTS WITH VANCOMYCIN RESISTANT ENTEROCOCCUS FAECIUM (VRE) COLONIZATION

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Vancomycin Variable Enterococci (VVE) linked to severe bloodstream infections in immunocompromised patients with Vancomycin Resistant Enterococcus faecium (VRE) colonization

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Introduction: Vancomycin Variable Enterococci (VVE) are a particularly devious emerging group of vancomycin-susceptible enterococci carrying vanA gene, that switch to a VRE phenotype under glycopeptide therapy. In this study, we aim to molecularly characterize the in vitro correlation between Vancomycin-Resistant Enterococcus faecium (VREfm) and Vancomycin-Variable E. faecium (VVEfm) in two different models, belonging to two patients both suffering from VREfm colonization and a VVEfm bloodstream infection. Materials and Methods: From both patients a colonizing VREfm (rectal swab) and an infectious VVEfm (blood sample) were isolated. For each strain antibiotic susceptibility testing (AST) was performed and clonality was evaluated via PFGE. The vanA clusters were reconstructed by both PCR and long-range PCR, against the Tn1546 prototype (acc. no. M97297). RT-qPCR were conducted to analyze vanA relative expression levels. Results: Strains from patient 1 (Model 1) - rectal VREfm1 and blood VVEfm1 – were clonal, AST also showed the same antibiotic susceptibility profiles (except for glycopeptides). Both strains carried an intact copy of the vanHAX operon and an IS1251 copy at the 3'-region of the vanZ gene; the regulatory region of both VREfm1 and VVEfm1 could not be amplified, suggesting that an extensive rearrangement has undergone. Expression level studies of the vanHAX core region showed that VVEfm1 had a significant reduction of the expression levels compared to the VREfm1 clone. Strains from patient 2 (Model 2) (rectal VREfm2 and blood VVEfm2), genotypically did not confirm clonality, which was foreshadowed by differences in AST profiles. Characterization of the Tn1546 element showed that both VREfm2 and VVEfm2 lacked the entire mobility and regulatory region upstream of vanH gene. VVEfm2 had a copy of the IS1251 inserted into the vanH gene, unlike VREfm2, while the downstream regions were intact. Similarly to

model 1, VVEfm2 showed a significant relative reduction in the vanHAX expression levels compared to VREfm2. Discussion and conclusions: we suppose that alterations in the regulatory region of the element are responsible for the reduced vanHAX gene expression. Allegedly, in model 1, VREfm1 isolate translocated to the bloodstream under antibiotic treatment, where rearrangement happened that turned the strain into a VVE. In model 2, we hypothesized that the Tn1546 has been horizontally transferred and rearranged (before or after the event) from the colonizing VREfm2 to the infectious strain, which acquired the VVE phenotype.

157 - ISSUES IN CEFIDEROCOL TESTING COMPARING COMMERCIAL METHODS TO BROTH MICRODILUTION IN IRON DEPLETED MEDIUM. ANALYSES OF THE PERFORMANCES, ATU AND TRAILING EFFECT ACCORDING TO THE EUCAST 2024 GUIDELINES

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Issues in cefiderocol testing comparing commercial methods to broth microdilution in iron depleted medium. Analyses of the performances, ATU and trailing effect according to the EUCAST 2024 guidelines.

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Introduction: Cefiderocol, a siderophore cephalosporin, exhibits activity against Gram-negative bacteria. However, its novel nature and unique testing requirements present challenges for in vitro evaluation. Current commercial methods and guidelines for cefiderocol testing are undergoing assessment due to these complexities. This study aims to evaluate the performance of ComASP®, MIC test strips (MST), and disk diffusion (DD) methods against the gold standard of broth microdilution in Iron Depleted Cation Adjusted Mueller Hinton broth (ID-BMD), focusing on the critical role of Area of Technical Uncertain (ATU) and trailing effect, considering the evolution of the EUCAST guidelines.

Methods: Cefiderocol resistance profiles of 131 Gram-negative isolates (47 Enterobacterales, 25 A. baumannii, 9 S. maltophilia, and 50 P. aeruginosa) collected in September-October 2023 at the Policlinico Hospital in Catania were assessed using commercial and gold standard methods. The study evaluated categorical agreement (CA), essential agreement (EA), biases, percentage of major errors (ME), very major errors (VME), and the proportion of DD tests falling within the ATU. Results were interpreted according to the 2024 EUCAST guidelines, with ATU analyses comparing old and new guidelines.

Results: No cefiderocol resistance was observed by ID-BMD except for one K. pneumoniae isolate. The MIC90 for S. maltophilia was 0.5 mg/L, while for A. baumannii, it was 8 mg/L (without considering trailing effect) and 32 mg/L (considering complete growth inhibition by ID-BMD). CA and EA between ComASP and ID-BMD were 97% and 68% respectively for Enterobacterales, with 27% inferior biases and 2% VME. DD showed 72% CA with new EUCAST breakpoints and 93% with old guidelines, with 38% and 34% strains within ATU respectively, and 21% ME (no VME detected). For P. aeruginosa, CA

was 100%, with EA of 88% and 50% between ID-BMD and ComASP or MST with 100% or 20% superior biases observed, respectively. EA for A. baumannii was 56% (with only inferior biases) ignoring trailing effect, and 64% (still with inferior biases) considering complete growth inhibition.

Discussion and conclusions: This study provides insights into cefiderocol resistance epidemiology, indicating low resistance among Enterobacterales and P. aeruginosa. Findings suggest that DD overestimates resistance in Enterobacterales, while MTS underestimate MIC in P. aeruginosa. Trailing effect significantly impacts MIC determination for A. baumannii. ATU warrants attention as new range values increase discordance between DD and ID-BMDMIC, leading to incorrect characterization of false resistant strains; indeed, almost all strains within ATU were susceptible by ID-BMD.

160 - FIRST DETECTION OF A PLASMID CO-CARRYING BLAVIM-1 AND MCR-9 IN A KLEBSIELLA GRIMONTII FROM AQUATIC ENVIRONMENT

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First detection of a plasmid co-carrying blaVIM-1 and mcr-9 in a Klebsiella grimontii from aquatic environment

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Introduction. Klebsiella grimontii is a newly identified emerging pathogen closely related to Klebsiella oxytoca. Plasmid-mediated carbapenem-resistance in K. grimontii has recently been reported although only in 3 clinical strains. We have detected the first carbapenem resistant K. grimontii strain from environment in Italy.

Materials and Methods. The K. grimontii was isolated from marine sediment by plating on MacConkey agar supplemented with ertapenem. Carbapenem resistance was confirmed by multiplex PCR for carbapenemase genes and MIC determination. Whole genome sequencing and the comparison with 1098 genomes available in NCBI were carried out. Genomes deposited as K. oxytoca and K. michiganensis were also included in the analysis considering the incorrect classification of many K. grimontii strain in the database.

Results. K. grimontii was ertapenem resistant (MIC 4 µg/ml) and showed the VIM carbapenemase-encoding gene. The strain belonged to the sequence type ST172. Whole-genome sequencing highlighted the presence of a 300-kb IncHI2/HI2A-Pst1 plasmid co-carrying blaVIM-1 and mcr-9. The blaVIM-1 gene was included in the In110 class 1 integron, while mcr-9 in a cassette bracketed by IS903 and ΔIS1R. This plasmid was highly similar to that carried by a clinical K. grimontii strain isolated in Switzerland but also similar to other Enterobacterales plasmids isolated from food and animal sources. The plasmid also carried: blaACC-1, sul1, aac(6')-Ib3, aadA1, aph(3'')-Ib, aph(6)-Id and catA1. Core-genome phylogenetic analysis indicated that except for an environmental strain isolated in Slovenia, the majority of the closely related genomic sequences were of clinical strains. In particular, the closest genome sequence was a clinical isolate collected from urine in the USA (interactive phylogenetic tree available at https://microreact.org/project/9zEMfAZKSdJd5PY6qBeXGj-kgrimontiiclosest).

Discussion and conclusion. The report of a new emerging MDR pathogen as K. grimontii from the environment poses a direct threat to public health. The presence of a plasmid typically associated with clinical strains in a marine isolate could reflect its spillover from human and/or animal reservoirs

following horizontal gene transfer events. The study also highlights that coastal seawaters might represent a niche of clinically relevant resistance genes and mobile elements that could spread undetected.

177 - ISOLATION OF COLISTIN-RESISTANT LOS-DEFICIENT ACINETOBACTER BAUMANNII IN SINGLE AND DOUBLE MUTANTS INACTIVATED FOR PROTEINS INVOLVED IN PEPTIDOGLYCAN BIOSYNTHESIS AND REMODELING

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Isolation of colistin-resistant LOS-deficient Acinetobacter baumannii in single and double mutants inactivated for proteins involved in peptidoglycan biosynthesis and remodeling.

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1. Introduction: The emergence and spread of antimicrobial resistance (AMR) is of extreme clinical relevance. Among the ESKAPE pathogens, Acinetobacter baumannii, is a nosocomial and community acquired Gram-negative bacterium often multi- or pan-resistance to antibiotics. Colistin, which targets the lipid A domain of the LPS/LOS of Gram-negatives bacteria, is the last-line resource for multidrug-resistant A. baumannii infections. Despite lipid A being essential for most Gram-negative bacteria, colistin-resistant LOS-deficient A. baumannii was reported to be isolated after colistin exposure in clinical strains producing low amounts of the class a penicillin-binding protein 1A (PBP1a) or delta-PBP1a mutants. In this work, we phenotypically characterized the A. baumannii AB5075 WT strain as well as single and double mutants defective in selected non-essential proteins involved in PG biosynthesis and remodeling and assessed their ability to generate colistin-resistant LOS-deficient variants. 2. Materials and Methods: The clinical isolate A. baumannii AB5075 strain, and its transposon-inactivated isogenic single and double mutants were characterized for growth, viability and morphology. In addition, their resistance profile to a panel of antibiotics, mainly targeting the cell envelope, was evaluated using the Epsilometric test (E-test) method and the broth microdilution method to determine the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal Concentration (MBC). In addition, a SDS/EDTA sensitivity assay was performed to detect mutant-specific phenotypes upon stress. Colistin-resistant LOS- mutants were isolated by plating the WT and mutants onto LB agar plates containing colistin and confirmed by replica-plating onto LB agar plates containing colistin or vancomycin. 3. Results: A. baumannii single mutants, lacking the genes encoding the penicillin-binding proteins pbp1a or pbp1b and the LD-transpeptidase ldtJ, displayed clear morphological defects, despite none of the mutants showing significant differences in growth and viability compared to the WT strain. On the other hand, Δpbp1a/ΔldtJ double mutants showed a dramatic phenotype, with elongated and bulged cells, supporting the notion that LdtJ is crucial in the absence of PBP1a, while Δpbp1b/ΔldtJ double mutants were similar to the single Δpbp1b mutants. A.

baumannii exhibited overall high sensitivity to SDS/EDTA treatment, with mutant-specific differences detectable already at low concentrations, and particularly evident for the double Δpbp1a/ΔldtJ mutants. Finally, the frequency of colistin-resistant LOS- mutants was highest for the double Δpbp1a/ΔldtJ mutants compared to the WT and the single Δpbp1a or ΔldtJ mutants, suggesting that, in the AB5075 background, either LdtJ is not required to develop colistin resistance or the double Δpbp1a/ΔldtJ mutants acquired suppressors mutations that allowed this. 4. Discussion and Conclusion: The results show a great aptitude of A. baumanni to survive the inactivation of genes involved in PG synthesis and remodeling without serious consequences. The ability to generate colistin-resistant LOS-deficient variants at higher frequency in the Δpbp1a and Δpbp1a/ΔldtJ mutants suggests that LOS can be dispensable in some A. baumanni genetic backgrounds but this likely requires PG synthesis and modifications to be re-programmed. Taken together, the results underline the importance of understanding how the different components of the cell envelope are interconnected to allow A. baumanni to grow and divide under physiological and stress conditions while coping with antibiotic resistance.

201 - LAST TRENDS IN ANTIBIOTIC RESISTANCE AND FUTURE MEASURES FOR ANTIMICROBIAL DIAGNOSTIC STEWARDSHIP IN AEROBIC VAGINITIS

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Last Trends in Antibiotic Resistance and Future Measures for Antimicrobial Diagnostic Stewardship in Aerobic Vaginitis

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Introduction

Aerobic vaginitis (AV), frequently linked with bacterial vaginosis, Candida, and parasitic infections, is a disruption of the vaginal microbiota commonly seen in fertile women between the ages of 15 and 44. This dysbiosis can lead to several complications including endometritis, cervicitis, urinary tract infections, premature births or late miscarriages. It also poses a risk factor for diseases such as cancer, diabetes, cardiovascular diseases, and infertility. The focus of this study is a five-year assessment of women in their reproductive years suffering from AV, identifying the primary bacteria and studying their pattern of antibiotic resistance. Early diagnosis and specific treatment are crucial elements of antimicrobial stewardship.

Materials and Method

Our study was conducted at the University Hospital of Campania "L. Vanvitelli" among women seeking healthcare in Naples, a metropolitan city of Campania, over the period from 2018 to 2023. We gathered clinical isolates from women aged 20 to 60 who were hospitalized in the Complex Operative Unit of Virology and Microbiology.

Results

Between January 2018 and January 2023, we analyzed 1951 vaginal swabs. During this period, 58.6% of the samples tested positive. The most frequently found strains were Escherichia coli (n = 153), Enterococcus faecalis (n = 149), Staphylococcus haemolitycus (n = 61), and Candida albicans (n = 87). For E. coli resistance to amoxicillin/clavulanic acid and ampicillin was expected to increase in 2021-2022. On the other hand, susceptibility rates to fluoroquinolones significantly dropped between 2019

and 2022. Among the gram-positive isolates, E. faecalis was found to be susceptible to vancomycin, linezolid, and teicoplanin. In the past two years, resistance rates to ampicillin, imipenem, and ciprofloxacin were 22% and 5%, respectively. For S. haemolyticus strains, no resistance was reported to vancomycin, linezolid, teicoplanin, and daptomycin; however, in 2019, all isolates were found to be methicillin-resistant. The susceptibility patterns for aminoglycosides and macrolides were reversed; gentamicin susceptibility rose in 2020 and 2021, while clindamycin and erythromycin showed high resistance rates from 2020 onwards (73% to 80%, respectively).

Discussion and Conclusions

The development of antimicrobial resistance is influenced by a variety of elements, such as the host, the infectious agent, and the surrounding environment. In an era where the pipeline for new antibiotics is drying up, it's crucial to prioritize antimicrobial diagnostic stewardship when diagnosing and treating conditions like aerobic vaginitis.

211 - RAPID PREDICTION OF FLUCONAZOLE RESISTANCE BY MACHINE-LEARNING ANALYSIS OF CANDIDA PARAPSILOSIS MALDI-TOF MASS PROFILES

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Rapid prediction of fluconazole resistance by machine-learning analysis of Candida parapsilosis MALDI-TOF mass profiles

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Introduction: Candida parapsilosis resistance to azoles has emerged in the last ten years rising the concern for the spread of clonal resistant isolates in the hospitals, particularly in Southern Europe, Latin America and Asia. The main mechanism involves ERG11p substitutions being Y132F the dominant one reported in several cases of invasive candidiasis. Coupling machine-learning approach and MALDI TOF analysis, the aim of the present study has been the assembly of a dataset of MALDI-TOF mass spectra for the algorithm training and its validation for the rapid detection (ten minutes vs 24 hours) of fluconazole resistant (FLZ-R) Candida parapsilosis isolates.

Materials and methods: Overall 204 C. parapsilosis mass spectra profiles, 77 FLZ-R or -intermediate and 127 -susceptible acquired by Autof MS 2600 mass spectrometer (Autobio) and previously characterized by sequencing of ERG11 gene were assigned to the positive or negative class respectively and used as training dataset. Clover MS Data analysis software was used for the machine learning approach using Support Vector Machine (SVM) classifier for the testing dataset classification. Seventy-four C. parapsilosis profiles were used for the testing dataset including an iced collection and routine diagnostic clinical samples retrieved from blood cultures. Susceptibility to fluconazole was determined by the Sensititre YeastOne antifungal panel ITAMYUCC (Thermofisher).

Results: SVM classifier was selected as the best-performing receiver operating curve (ROC of 0.80) to predict fluconazole resistance. In summary, 26 out of 37 (70.3%) C. parapsilosis isolates were correctly identified as FLZ-susceptible or -resistant, 4 strains were misidentified and 7 resulted undetermined.

Discussion and conclusions: C. parapsilosis candidemia outbreaks have been reported as a matter of concern in the fungi arena. Even if at its first steps and requiring improvement of the classification performance, MALDI-TOF mass spectra-based fluconazole resistance prediction by machine learning approach might enable rapid and low-cost resistance detection compared to the clinical microbiology laboratories standard of phenotypic resistance determination and has the potential to improve patient treatment decisions and antifungal stewardship.

218 - CEFIDEROCOL RESISTANCE IN KLEBSIELLA PNEUMONIAE NDM OR NDM/OXA-48-LIKE PRODUCING STRAINS

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Cefiderocol resistance in Klebsiella pneumoniae NDM or NDM/OXA-48-like producing strains

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Introduction: New-Delhi metallo-β-lactamase (NDM) producer Klebsiella pneumoniae is one of the most represented antibiotic-resistant Enterobacteriaceae worldwide for its capacity to harbour mobile elements codifying for different carbapenemases, leading to a challenging resistance pattern.

Materials and Methods: We selected 29 K. pneumoniae isolates collected from a multidrug-resistant (MDR) screening program conducted in Verona hospitals (Italy) between 2022 and 2023. Isolates were selected for their NDM production through phenotypic and molecular tests: NG-test® CARBA5 (NG-biotech, France) and Carba-NP rapid tests discriminated NDM production and co-harbouring with OXA-48-like. Carbapenems, ceftazidime and cefepime minimum inhibitory concentrations (MICs) were evaluated through the broth-microdilution method. At the same time, the four beta-lactam/beta-lactamases inhibitor combinations (BL-BLICs) were assessed through E-tests (Liofilchem, Italy). Cefiderocol was tested using the ComASP® kit (Liofilchem) and the disc diffusion method. End-point PCR for blaNDM and blaOXA-48-like genes was performed, and PCR products were purified (Qiagen, Germany) and sequenced (Eurofins, Germany) to discriminate carbapenemases variants. Isolates resistant to cefiderocol were evaluated through end-point PCR and newly designed primers, followed by purification and sequencing, for cirA gene mutations, a siderophore transporter associated with cefiderocol resistance when mutated.

Results: All isolates tested positive for NDM production and eight for OXA-48-like co-harbouring. MICs for cephalosporins and ertapenem tested fully resistant in all cases, while MICs for imipenem and meropenem tested resistant in all NDM-1 and OXA-232/OXA-48 co-harbouring isolates. In BL-BLICs and cefiderocol activity evaluation, we saw a particular pattern of resistance: NDM-1 plus OXA-232/OXA-48 isolates tested fully resistant to ceftolozane-tazobactam (C/T), imipenem-relebactam (I/R) and meropenem-vaborbactam (M/V), while susceptible to ceftazidime-avibactam (CZA) and cefiderocol; NDM-1 strains tested fully resistant to C/T but susceptible to all other BL-BLICs and to cefiderocol; NDM-5 strains tested fully resistant to C/T and CZA, and susceptible to I/R and M/V, with eleven isolates resistant to cefiderocol. Cefiderocol-resistant cirA gene sequences were challenging: we found seven different point mutations, with the W367G mutation considered deleterious. An IS6-family transposable element was inserted upstream of the cirA gene in four isolates.

Discussion and Conclusions: These results suggest that K. pneumoniae tends to modify its resistance pattern to respond selectively to cephalosporins or carbapenems, and cirA regulation plays a crucial role in cefiderocol activity.

229 - A FORMULATION OF LACTONASES AGAINST PSEUDOMONAS AERUGINOSA INFECTIONS: EFFECT ON VIRULENCE FACTORS IN VITRO STUDIES AND IN ANIMAL MODEL

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A formulation of lactonases against Pseudomonas aeruginosa infections: effect on virulence factors in vitro studies and in animal model

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Introduction

The quorum-sensing (QS) circuit in gram-negative bacteria, like P. aeruginosa (PAO1), coordinates the regulation of virulence factors and biofilm formation through mainly two specific acyl-homoserine lactones (AHLs), 3OC12-HSL and C4-HSL. However, the role of QS-controlled biofilm formation in clinical settings has emerged due to increasing antibiotic resistance. Therefore, there's a critical need to find new compounds with anti-biofilm or anti-QS activities. One approach is quorum quenching (QQ), where QS molecules can be disrupted by using exogenous AHL-degrading lactonases. Phosphotriesterase-Like Lactonases (PLLs), initially studied by our group for phosphotriesterase activity, have been found to target lactones effectively. AhlA, a PLL from R. erythropolis, is particularly promising as it hydrolyzes 3OC12-HSL and C4-HSL, inhibiting PAO1 biofilm formation. To further enhance this effect, an enzymatic formulation composed of thermostable and human lactonases has been developed, comprising three enzymes with high lactonase activity. This enzyme mixture was designed to inhibit biofilm formation in gram-negative bacteria, particularly in the treatment of infected wounds, as tested in vitro studies and in animal model. This strategy aims to reduce the need for antibiotics, thereby lowering the risk of the increasing of antibiotic resistance.

Materials and Methods

For each enzyme we performed the expression, purification, and characterization. The purified enzymes were tested, alone or in a mix, for their ability to inhibit the PAO1 biofilm formation, by using the crystal violet assay. This formulation was tested in wound healing assays on immortalized HeLa cells in vitro and used to treat infected skin wounds in vivo model CD1 mice.

Results

The enzyme formulation was able to reduce the PAO1 biofilm of 60%. The reduction in biofilm formation was higher when the formulation was used, compared to the single enzymes tested separately, even if the total concentration was lower, due to the synergistic effect of the three

lactonases. Treatment of mice infected wounds with the enzyme mix led to a faster wound closure respect to the controls, and a lower bacterial infection.

Discussion and Conclusions

The enzyme mix was designed to include the enzymes with complementary characteristics:

High specificity on the main QS signal, 3OC12-HSL and C4-HSL.

High stability over time.

Resistance to common disinfectant (H2O2).

Encouragingly, positive results were achieved, showing that this formulation can be used for the treatment of acute PAO1 infections. It will also be tested in other conditions, such as chronic infections (as in cystic fibrosis patients) and for the immobilization on solid surfaces (as medical and surgical devices).

237 - CHARACTERIZATION OF HIV-DNA MINORITY MUTATIONS IN LONG-TERM INFECTED PAEDIATRIC INDIVIDUALS

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Characterization of HIV-DNA minority mutations

in long-term infected paediatric individuals

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Introduction

Next-generation sequencing (NGS) of HIV-DNA can provide more sensitive information for individuals with a low or undetectable viremia and/or no previous genotype. The aim of the study was to better understand the impact of HIV-1 minority and APOBEC-related mutations (APO-Ms) to guide the best personalized antiretroviral treatment (ART) in paediatric population.

Materials and methods

From August 2022 to March 2023, NGS was performed on HIV-DNA positive blood samples belonging to 22 individuals, mostly with HIV-vertical transmission referred at IRCCS Bambino Gesù Children’s Hospital. The presence of minority resistance mutations (mRMs) (frequency: 5-20%) and APO-Ms was evaluated. For 12 individuals with available information, we compared mutational profiles obtained in NGS with those by using Sanger sequencing.

Results

The participants were mainly male (13; 59.1%) with a median (IQR) age of 19 (16-25) years. Eighteen (81.8%) individuals had an undetectable or <200copies/mL of HIV-RNA, the remaining 4 children, had a median (IQR) HIV-RNA of 43272 (16373-488497) copies/mL. The HIV-DNA was detectable for all participants with a median (IQR) of 1435(215-4819) copies/106CD4+T-cells.

Ten individuals (45.5%) received an early ART with a median duration of 19 (IQR:10-23) years. All participants (22; 100%) received Nucleoside Reverse Transcriptase inhibitors (NRTI) regimen, 72.7% (N=16) Protease-Inhibitors (PI) and 40.9% (N=9) Non-Nucleoside-RTI (NNRTI) regimen.

12 (54.5%) individuals had at least one HIV-1 major RMs in HIV-DNA. NRTI, NNRTI and PI resistances were present in 58.3%, 50% and 8.3% of cases, respectively. Five (41.7%) participants were detected to harbour at least one HIV-1 mRMs, mainly localized in RT followed by Protease region. Most samples contained APOBEC-related mutations (19; 86.4%) and 59.1% of samples (14/22) showed at least one APO-related stop codon, commonly detected as minority species.

No accumulated mutations were identified when compared NGS results with those of historical HIV-RNA genotypes.

Conclusions

These results could guide toward the characterization of unique population, with a long history of ART-treatment and clinical characteristics different from adults. The use of NGS allowed us to analyse in-depth the presence of minority and APOBEC-related mutations in HIV-DNA, more or less associated with resistance.

264 - IN VIVO EMERGENCE OF CEFIDEROCOL RESISTANCE IN PSEUDOMONAS AERUGINOSA FOLLOWING PROLONGED TREATMENT AT SAN MARTINO POLICLINICO HOSPITAL (HSM), GENOA, ITALY

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In vivo Emergence of Cefiderocol Resistance in Pseudomonas aeruginosa following prolonged treatment at San Martino Policlinico Hospital (HSM), Genoa, Italy

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Introduction

The spread of MDR P. aeruginosa has become a threat for the healthcare system, since resistance to first-line antipseudomonal agents, as ceftazidime/avibactam (CZA), ceftolozane/tazobactam (C/T), but also to carbapenems and cefiderocol (FDC), can rapidly emerge. Recently, cases of P. aeruginosa resistant to these agents have been reported, either due to mutations in functionally-diverse resident genes (e.g. coding for the resident Amp-C β-lactamase, blaPDC, porins and regulators of efflux pumps, and siderophore receptors involved in FDC uptake) or to acquired genes encoding metallo- (e.g. VIM-/NDM-types) and/or serine-β-lactamases (e.g. PER-, GES-, SHV-types). Here, we report a case of CZAR/C/TR P. aeruginosa developing resistance to FDC in a patient during treatment.

Materials and methods:

Identification and antibiotic susceptibility testing (AST) were performed with Vitek MS MALDI-TOF and VITEK2 system, respectively. Carbapenemases production was assessed by lateral flow Immunochromatography (LFIA), RT-PCR and mCIM assays. C/T and FDC testing was performed by E-test and by Kirby Bauer disk-diffusion, respectively. Genomic DNA was subjected to whole genome sequencing (WGS) with Illumina NovaSeq.

Results

Three P. aeruginosa isolates (PSA1, broncoaspirate; PSA2, sputum: PSA3, pharyngeal swab), sequentially collected from a patient exposed to prolonged treatment with FDC, were studied. All tested resistant to piperacillin/tazobactam, cephalosporins, including CZA and C/T, and carbapenems. PSA2 and PSA3, collected 31 and 41days apart since the treatment with FDC ended, exhibited reduced susceptibility to FDC (22, 19 mm vs 31 of PSA1). LFIA and RT-PCR did not reveal any carbapenemase in all strains, consistent with mCIM results. Analysis of WGS data revealed i) a non-functional oprD gene and mutations affecting the pseudomonas-derived cephalosporinase (PDC) regulator ampD (H71*) in all strains; ii) mutations in the PDC β-lactamase, evolving from PDC-36 (PSA1) to PDC-427 (PSA2, PSA3); iii) mutations in fptA (L24P) and pir (A133*), coding for siderophore receptors involved in FDC uptake.

Discussion and conclusion

FDC represents a promising treatment option against DTR/CR P. aeruginosa ¸ being not impacted by β-lactam resistance mechanisms commonly encountered in this species. Although resistance rates currently appear to be low, on-treatment emergence of FDC-resistant isolates has been reported, raising major concerns due to limited therapeutic alternatives. In our case, the prolonged FDC exposure (21 days) likely contributed to a stepwise selection of mutant isolates developing resistance. Further studies are needed to characterize the specific contribution of detected alterations to the FDC resistance phenotype.

267 - DECODING MOLECULAR MECHANISMS UNDERLYING DAPTOMYCIN RESISTANCE IN ENTEROCOCCUS FAECIUM CAUSING BLOODSTREAM INFECTIONS

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Decoding molecular mechanisms underlying daptomycin resistance in Enterococcus faecium causing bloodstream infections

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Introduction

The high prevalence of vancomycin-resistant Enterococcus faecium (VRE) in Italy, reaching 30.7% in 2022 and 26.9% in 2023 in bloodstream infections according to antibiotic-resistance surveillance data of Italian National Institute of Health's (AR-ISS), has prompted over last years the monitoring of isolates resistant to second-line drugs, such as daptomycin (DAP), that could silently spread within the healthcare setting. The aim of this study was to investigate the epidemiology and molecular basis of DAP resistance in clinical isolates of E. faecium collected at the IRCCS Polyclinic Hospital San Martino (HSM), Genoa.

Materials and Methods

Analysis of retrospective laboratory data (2021-2023) was done to define the local epidemiological background concerning resistance to vancomycin and DAP in E. faecium causing bloodstream infections. Identification and antibiotic susceptibility testing (AST) were done with VITEK MS MALDI-TOF and VITEK2, respectively. AST results were interpreted according to the EUCAST breakpoints (v.14). Isolates with MIC of DAP >8 mg/L (DAP-R) were subjected to whole genome sequencing (WGS) with Illumina NovaSeq. Sequence analysis was performed using bioinformatics tools: BLAST, AMRFinder, MLST, and QUAST.

Results

The local prevalence of VRE was 49% (106/217) in 2021, 36% (55/154) in 2022, and 39% (71/183) in 2023. DAP-R isolates were only detected among vancomycin-susceptible E. faecium (VSE), with a prevalence of 0.7% (1/154) in 2022 and 1.1% (2/183) in 2023. The three isolates, namely E45/22 (A), E1098/23 (B) and E1697/23 (C) were selected for phenotypic and molecular characterization. MIC values for DAP (mg/L) were 64 for A, 24 for B and 8 for C. According to WGS, isolates belonged to ST117 (A), ST80 (B) and ST262 (C), and carried mutation in genes associated with DAP-R, including: cls (R211Q), coding for a cardiolipin synthase, (detected in A); cls (N13T), liaR (W73C) and liaS (T120A),

coding for a transcriptional regulator and a sensor histidine kinase, respectively (identified in B); liaF (C216Y, I148V, L80F, V111I, V235I, V78A and Y47H), carrying previously uncharacterized mutations (identified in C).

Discussion and Conclusions

Although at low frequency, DAP-R has been globally recognized in VRE/VSE isolates. In this study, the DAP-R phenotype was due to alterations in liaFSR or cls housekeeping genes, reinforcing the role of these targets in resistance to DAP. Notably, unlike previously described DAP-R E. faecium, alterations simultaneously affecting all molecular targets associated with DAP-R and novel mutations in liaF were reported in two strains, respectively. Present results add to existing research on the molecular mechanisms underlying DAP-R in E. faecium.

270 - SYNERGISTIC POTENTIAL OF ESSENTIAL OILS WITH ANTIFUNGALS AGAINST RESISTANT CANDIDA AURIS CLINICAL ISOLATES.

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Synergistic potential of Essential Oils with antifungals against resistant Candida auris clinical isolates.

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Introduction. The appearance of pathogenic fungi is not a rare event. In recent decades, their occurrence is rising as a worldwide issue in human health, especially if we consider the limited number of antifungal drugs available for their treatment. Between these, the emergence of Candida auris has been rapid and overwhelming due to its resistance to various antifungal medications such as azoles, polyenes, and echinocandins. The most promising choices compared to conventional treatment of microbial infections are plant products, such as essential oils (EOs) known to be effective against bacterial and fungi. In the present study, the antifungal activities of fifteen EOs alone and in combination with antifungal agents were tested against clinical strains of C. auris.

Materials and Methods. The broth dilution method for determining the minimum inhibitory concentration (MIC) was used to evaluate the effect of both antifungals and EOs against the 23 clinical isolates of C. auris. Thereafter, the disc diffusion test was settled up to highlight the anti-C. auris efficiency of the most effective four antifungals – caspofungin, micafungin, fluconazole and 5-flucytosine and EOs towards two selected strains, isolated from a deep systemic infection and a cutaneous colonization. Finally, the interaction between the antifungal drugs and the EOs with the lowest MICs was further investigated using checkerboard assay.

Results. The yeasts displayed a variable profile of susceptibility/resistance to various antifungals. All yeasts were resistant to fluconazole (MIC >128 µg/ml). The data demonstrated that EOs inhibited the growth of C. auris, with MIC values ranging from 0.03 to 0.5% for efficacious cinnamon, clove bud, geranium, lemongrass, mentha of Pancalieri and thyme EOs and the inhibition halo further confirmed this pattern. A synergistic action towards C. auris was those highlighted for micafungin and cinnamon or clove bud or geranium or lemongrass or thyme EOs, with fluconazole and mentha of Pancalieri EO, and with 5-flucytosine and mentha of Pancalieri EO, but depending on the clinical strain.

Discussion and Conclusions. The here reported in vitro results indicate that EOs were able to inhibit the growth of fluconazole resistant C. auris clinical isolates and the checkerboard experiments demonstrated – at various extent – that the combination of an antifungal drug with EOs can potentiate the anti-C. auris activity reaching a synergic effect. EOs display antifungal features alone, and they might boost the effectiveness of traditional drugs that could be impaired in their activity towards resistant strains. In this context, the use of an association could let to a reduction in the dose of the individual components and indeed limiting side effects.

POSTER presentation

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282 - CLINICAL UTILITY OF GENOTYPIC DRUG-RESISTANCE TEST IN IMMUNOCOMPROMIZED PATIENTS WITH CYTOMEGALOVIRUS REFRACTORY INFECTION

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CLINICAL UTILITY OF GENOTYPIC DRUG-RESISTANCE TEST IN IMMUNOCOMPROMIZED PATIENTS WITH CYTOMEGALOVIRUS REFRACTORY INFECTION

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Introduction. The study evaluates the clinical utility of genotypic test for cytomegalovirus (CMV) drug resistance (DR) in immunocompromised patients with CMV refractory infection.

Material and methods. Ninety-seven samples (91 whole blood-WB, 1 cerebrospinal fluid-CSF, 3 bronchial aspirate-BAS, 1 rectal swab, 1 vitreous humor) from 94 episodes of refractory CMV infection occurred in 70 patients (33 recipients of hematopoietic stem cell transplant, HSCT; 28 solid organ transplant, SOT; 2 with autoimmune disease, AD; 4 with AIDS and 3 with hematological disease, HD) were tested. The UL97, UL54, UL56, UL89 and UL51 viral genes were analyzed by Sanger sequencing to

identify valganciclovir/ganciclovir (GCV), foscarnet (FOS), cidofovir (CDV), maribavir (MBV) and letermovir (LTV) resistance-associated mutations (r).

Results. CMV-DR mutations were detected in 34/94 (36.2%) episodes (27 patients: 12 CSE, 12 SOT, 1 with AIDS, 1 with HD and 1 with AD). For 27/34 (79.4%) cases, single or multiple DR mutations on UL97 gene were found: 26 associated with GCVr and 1 with MBVr. Therapy was switched to FOS (n=19), CDV (n=1) or LTV (n=1). One patient developed CMV myocarditis. Mutations associated to multidrug resistance were detected in 4/34 (11.8%) episodes: GCVr and CDVr (n=2); GCVr and FOS (n=1); GCVr, CDVr and FOS (n=1). Treatment was shifted to FOS (n=2), MBV (n=1) and anti-CMV Ig with leflunomide (n=1). One patient developed CMV retinitis. Finally, single LTVr mutations in UL56 gene were detected in 3/34 (8.8%) cases receiving off-label LTV treatment, that were switched to GCV. Compartmentalized CMV-DR infections were documented in 2/34 cases: CMV strains associated to GCVr were found in a CSF sample from 1 AIDS patient with CMV-encephalitis and in a BAS sample from 1 lung transplant recipient with respiratory signs. For both, treatment was switched to FOS. No CMV-DR mutations were identified in the remaining 60/94 (63.8%) episodes (53 patients: 26 CSE, 21 SOT, 3 with AIDS, 2 with HD and 1 with AD) and in 25/60 (42%) cases the second line therapy was avoided. Among them, 2 cases developed CMV-related chorioretinitis and gastroenteritis.

Discussion and conclusions. Results showed i) the clinical utility of CMV-DR test to identify appropriate antiviral therapy limiting high toxicity drugs administration ii) the relevance to test representative samples in cases of compartmentalized CMV-DR strains.

287 - THE ROLE OF DRINKING MINERAL WATER IN THE SPREAD OF ANTIBIOTIC RESISTANCE AND VIRULENCE GENES

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The role of drinking mineral water in the spread of antibiotic resistance and virulence genes

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Introduction: Antibiotic resistance surveillance is an essential tool for studying and describing the emergence, spread, and trend of antimicrobial resistance. Resistant bacteria, virulence genes, resistant genetic elements, antibiotic metabolites, and drug residues pass into wastewater and water treatment plants, contaminating waterways such as lakes and oceans, irrigation, and drinking water that reaches the final consumer. Recently, not only natural surface water and wastewater, but also drinking water, especially water treated with purification processes, have been considered as “potential reservoirs” of antibiotic-resistant bacteria and genes. Microorganisms can be removed after the treatment process, but their genetic determinants of resistance with transforming power could remain and be transmitted to the consumer and his or her microbiota. To date, 90.3% of Italians drink mineral water. To assess the role of drinking water in the spread of antibiotic resistance, the aim of the present study was to evaluate the microbial composition, antibiotic resistance and virulence genes, in commercially bottled water.. Materials and Methods: For the study, 100 liters of four different brands of bottled water were analyzed. After filtration, using a six-way filtration ramp with 0.22-micron filters, DNA extraction was performed from the filtrate using a dedicated extraction kit. Extracted DNA was sequenced by shotgun sequencing using the Illumina NextSeq500 platform, and subsequent bioinformatics analysis allowed the characterization of the microbiota associated, and of specific genes related to bacterial antibiotic resistance and virulence. Results: A prevalence of the phylum Proteobacteria was shown for all but one sample. The antibiotic resistance genes identified were: genes for Beta-Lactamase synthesis, such as CblA-1, CfxA2 and PDC-5; genes for efflux pump synthesis (NBU2, Omp, PmpM, EmrE, MepA); and genes related to resistance of other antibiotics, such as aminoglycosides (APH(3')-IIb), tetracyclines (Tet) and phosphomycin (FosA). In addition, virulence factors identified by the analysis were genes characteristic of bacterial species considered pathogenic, as Pseudomonas aeruginosa, Mycobacterium tubercolosis, Bordetella pertussis and Brucella melitensis. Discussion and Conclusions: Study results indicated the presence in drinking water of genes related to antibiotics resistance and virulence factors, also identifying drinking water, conventionally defined as "microbiologically pure," as another possible reservoir. This study could lead to expanding the parameters for assessing drinking water quality and its role in the phenomenon of the spread of antibiotic resistance.

T08 EPIDEMIOLOGIA

5 - PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIAL PATHOGENS IN COMMUNITY URINARY TRACT INFECTIONS ISOLATED IN DIAGNOSTIC INSTITUTE VARELLI OF NAPLES

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Prevalence and antimicrobial resistance patterns of bacterial pathogens in community urinary tract infections isolated in diagnostic Institute Varelli of Naples

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INTRODUCTION: Urinary tract infections (UTIs) are the second most common cause of bacterial infection, after those of the respiratory and are considered a public health threat given the mounting rates of antibiotic-resistance. UTIs are among the most common bacterial infections acquired in the community and in hospitals. Treatment of UTIs with antibiotics reduces symptoms and bacteriuria, but also selects for resistant uropathogens. Uropathogens are more and more resistant to currently available antibiotics, so that new strategies for managing UTIs are needed. In this study, we estimated the frequency of UTI-associated pathogens and their antimicrobial resistance profiles.

MATERIALS AND METHODS: Our study shows data collected on patients of the Diagnostic Institute Varelli in Naples in four years (January 2019 – December 2022). The samples were screened using the automated system ALFRED60 (Alifax). Positive urine samples were sown on CHROMagar Orientation Medium (BD Diagnostic System) and incubated overnight at 37°C. Bacteriuria was defined by the number greater than 104 CFU/mL and by a monomorphic growth. Bacterial identification and antibiotic susceptibility testing were performed using Matrix assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) and MicroScan WalkAway plus (Beckman Coulter).

RESULTS: Among the 106346 studied patients, 22676 (21,32%) and 83670 (78,68%) were positive and negative for microorganism growth, respectively.

Of 22676 positive patients, 16901 (74,53%) females and 5775 (25,47%) males were identified. The highest incidence of positive subjects was recorded in the elderly (>61 years).

Among the uropathogenic strains, 22093 (97,43%) were Gram-negative and 583 (2,57%) were Gram-positive. The most isolated Gram negative strains were E.coli (69,85%) of which 20,85% were strains producing extended-spectrum β-lactamases (ESBL), K. pneumoniae (13,89%), P. mirabilis (5,96%) and P. aeruginosa (1,82%). The most frequent Gram-positive strain was E. faecalis (1,43%). Gram-negative bacteria showed highly resistant to Ampicillin with the increasing resistance of Ciprofloxacin and

Levofloxacin, which are commonly used as empirical treatment in most of UTIs, whereas Gram-positive bacteria were highly resistant to Erythromycin.

DISCUSSION AND CONCLUSIONS: The aim of the study is to define the prevalence and the distribution of urinary tract infections and their causes. In accord with other epidemiological study, there's a prevalence of Gram negative isolates expecially E. coli, but also K. pneumoniae and P. mirabilis. This finding was in agreement with the common knowledge about the causative agents of UTI and the relative profile of resistance.

6 - SURVEILLANCE OF NDM-PRODUCING KLEBSIELLA PNEUMONIAE ISOLATES: PHENOTYPIC AND MOLECULAR CHARACTERIZATION

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Surveillance of NDM-producing Klebsiella pneumoniae isolates: phenotypic and molecular characterization

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Introduction

Klebsiella pneumoniae-producing NDM carbapenemase (NDM-Kpn) is a serious nosocomial pathogen causing a variety of infections, including pneumonia, urinary tract infections, and bloodstream infections. Treatment options are particularly limited, given their intrinsic and acquired resistance ability. Recently NDM-Kpn has aroused increasing attention, because of the identification of different outbreaks in Italy. Here, we report a retrospective study of investigating the molecular epidemiology of clinical K. pneumoniae isolates in the Latium region, Italy.

Materials and Methods

Between January 2019 and August 2023, 80 K. pneumoniae strains were isolated from clinical samples of patients treated from different hospitals in Latium region. Species identification and antimicrobial susceptibility were obtained by MALDI-TOF and Phoenix system (Bruker Daltonics, Germany; Becton Dickinson Diagnostic System, Sparks, US). MIC breakpoints were interpreted according to EUCAST recommendations. Carbapenemase NDM production was confirmed by immunochromatographic assay (NG-test Carba, NG Biotech). Whole Genome Sequencing (WGS) was performed by Illumina Miseq (Illumina, Inc., US). Antimicrobial resistance and virulence genes were identified through in silico analysis using the ResFinder and Kleborate tools from the NGS data. Typing was conducted by traditional seven housekeeping genes-based MLST and NGS-based core genome MLST (cgMLST) (Ridom SeqSphere+ software version 2.1, Ridom GmbH, Münster, Germany).

Results

All 80 NDM-Kpn isolates showed an MDR profile; the blaNDM-1 gene was detected in 68/80 isolates and blaNDM-5 in 12/80 isolates. Additional ESBLs and carbapenemase genes such as blaCTX-M-15, blaKPC-3, blaOXA-48, blaCMY-6 and blaDHA-1 were also identified. 14 different Sequence Types (STs) were detected: ST147 (nr.32 isolates), ST11 (nr.16), ST395 (nr.7), ST15 and ST383 (nr.5), ST512 and ST1805 (nr.4), and one isolate for ST17, ST23, ST29, ST234, ST307, ST117 e ST4853. Clonal relationships within the STs, using the cgMLST scheme, showed the presence of 16 complex types (CT). Interestingly, 24/80 isolates (13 ST147 KL64, 7 ST395 KL2, 3 ST15 KL112 and 1 ST23 KL57) showed a relevant virulence score carrying ybt, ICEKp, iuc and rmp genes.

Discussion and Conclusion

The emergence of NDM-Kpn in Italy poses a serious threat to public health. This study highlighted the key role of surveillance in tracing the NDM-Kpn strains circulating in Italy, adding further insight into their molecular features. It is essential to enhance surveillance and investigation using NGS to identify high-risk clones, implementing effective control measures to prevent further spread.

21 - PREVALENCE OF COLONIZATION WITH CRE, VRE AND ESBL STRAINS OVER THREE YEARS: A SINGLE CENTER EXPERIENCE.

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Prevalence of colonization with CRE, VRE and ESBL strains over three years: a single center experience

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INTRODUCTION

Carbapenem-resistant Enterobacterales (CRE), extended-spectrum beta-lactamase-producing bacteria (ESBL) and vancomycin-resistant enterococci (VRE) represent one of the primary causes of healthcare-associated infections. This study aims to evaluate the prevalence of their colonization in a single center over the last three years.

MATERIALS AND METHODS

This retrospective observational study included 12,485 rectal swabs from 7,750 patients collected for active surveillance at the Microbiology and Virology Unit of the IRCCS Fondazione Ca' Granda, Ospedale Maggiore Policlinico of Milan between January 2021 and December 2023. All the bacteria grown on the CHROMID® CARBA, ESBL, VRE (Biomerieux®) selective media were identified using MALDI-TOF mass spectrometry (Biomerieux®). ESBL, VRE and CRE were further confirmed by a lateral flow immunoassay NG-Test CTX-M, by Disc Diffusion Method for Vancomycin, and by the NG-Test CARBA-5 (Ng-Biotech, CARBA-5) or by molecular assay (Xpert CARBA-R, Cepheid), respectively. Chi-squared test for trend was used to estimate significant changes in CRE, ESBL, and VRE over three years.

RESULTS

Patients were mainly male (60.9%), with a median age (IQR) of 68 (55-79) years and mainly referred to Medicine wards (53.3%).

The overall prevalence of ESBL, VRE and CRE colonization upon admission was 34.2% (n=4,271), with a progressive increase from 34.2% in 2021 to 36.8% in 2023 ($p<0.0001$). The higher prevalence was related to ESBL and VRE colonization (18.2% and 17.2%, respectively), followed by CRE colonization (6.1%). Of note, the prevalence of all three resistance mechanisms significantly increased over time (ESBL: 16.5% in 2021 to 20.7% in 2023, $p<0.0001$; VRE: 17.1% in 2021 to 18.4% in 2023, $p=0.025$; CRE: 4.9% in 2021 to 6.2% in 2023, $p=0.0009$).

Regarding CRE, KPC was the most frequent enzyme detected (3.0%, n= 369), followed by NDM (2.0%, n=251) and VIM (0.90%, N=112). Among these, VIM was the only CRE significantly increasing from 0.1% in 2021 to 1.3% in 2023 ($p<0.0001$).

Combined resistance was detected in 5.6% of rectal swabs (n=700) and was mostly related to ESBL+VRE colonization (3.1%, n=366) followed by VRE+CARBA (1.1%, n=133). The prevalence of combined resistance slightly increased over time, mostly due to VRE+CARBA combination (0.8% in 2021 to 1.5% in 2023, $p=0.0046$).

DISCUSSION AND CONCLUSION

This single-center study highlights the increased prevalence of ESBL, VRE and CRE colonization over time. Even if KPC remains the most frequent CRE detected, VIM was the only CRE significantly increasing its prevalence over time. This increase can be considered as a hallmark for the upsurgence of Metallo-β-lactamases to predominant resistance enzymes among CRE.

32 - PROVIDENCIA NIGERIAE: A NOVEL SPECIES WITHIN PROVIDENCIA SPP. FROM AFRICAN CONTINENT

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Providencia nigeriae: a novel species within Providencia spp. from African continent

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Introduction: Providencia spp. are motile gram-negative bacilli of the Family Morganellaceae, commonly found in soil, sewage, and manure. Among the species included in the above genus, relevant human opportunistic pathogens, such as P. stuartii, P. rettgeri, P. rustigiani and P. huaxiensis, are included. The aim of the study was to describe Providencia nigeriae, a novel species of Providencia (U32) collected in Nigeria from human source. Materials and Methods: Tentative species identification and antibiotics susceptibility were assessed through MICROSCAN Autoscan4, (EUCAST 2023 breakpoints). Whole-genome sequencing (WGS) of the strain was achieved through NovaSeq6000 (Illumina). Genome-based taxonomy was called using the all-in-one platform TYGS. The average nucleotide identity with other species within the same genus was obtained using FastANI tool. Resistome and plasmid replicon content were determined through ResFinder 4.1 and PlasmidFinder. Results: As to the metabolic profile, U32 ferments lactose and metabolizes glucose, inosine, urea, adonitol, tropodithietic acid, citrate, OF/Glucose and esculin. It owned catalase, but not oxidase activity. U32 showed a multi-drug resistant profile, being resistant to cephalosporins, gentamicin, quinolones, meropenem/vaborbactam, tobramycin and trimethoprim/sulfamethoxazole. The WGS analysis revealed a 3,971,369 bp genome length and an overall content of 4,349 genes (Gene average length= 913.17 bp). The N50 was over the literature threshold of N50>5000 bp (N50= 282,957 bp), as well as for the depth coverage with a threshold of 50X (depth= 103X). The contamination ratio was below the threshold <10% (c= 3%). The GC content in gene region was 41.70%, while in intergenic region 33.09%. The analysis with FastANI showed value below the same-species cutoff of 95%, revealing the highest identity (84.94%) with P. rettgeri species. The dDDH analysis confirm the same results, showing values below 70%. The 16S rRNA analysis pointed out a common evolutionary route with P. vermicola, while the genome blast distance phylogeny highlighted similarities with Shewanella bicestrii. The resistome comprises resistance genes coding for efflux pumps (rsmA, adeF, qacEdelta1),

aminoglycoside (aph(6)-Id, aph(3")-Ib, aac(6')-Ib-cr4, aac(3)-Ile, ant(3")-Ila), sulphonamide (sul1, sul2), trimethroprim (dfrA6, dfrA1), fluoroquinolone (qnrVC4, qnrD1), beta-lactams (blaVEB-5, blaTEM-1, blaCTX-M-15, blaCRP, PBP3), tetracycline (tet(59)) and phenicol resistance (floR). U32 resulted enriched with plasmids IncU and ColC. Discussion and Conclusions: Here we described a novel species of Providencia, named P. nigeriae, from Nigeria. Further investigation will be conducted to evaluate 16S rRNA on a Sanger based and to check the ARGs transferability.

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49 - HUMAN PAPILLOMAVIRUS (HPV) DETECTION AND GENOTYPING AMONG WOMEN WITH A RECENT ABNORMAL CERVICAL CYTOLOGY RESULT

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Human Papillomavirus (HPV) detection and genotyping among women with a recent abnormal cervical cytology result

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Introduction: Human Papillomavirus infection has been shown to be the cause of cervical cancer. This infection is very common among the human population but luckily only a small proportion of cases lead to precancer and cancer lesions. International guidelines recommend using high-risk (hr) HPV detection as primary test for cervical cancer screening, especially for women aged older than 30yrs. Only persistent hrHPV infections have been associated with the progression of cervical dysplasia. This ongoing study aims to study the role of hrHPV genotyping to distinguish clinically relevant from transient infections.

Materials and Methods: Cervical samples have presently been collected from 351 women with recent cervical dysplasia enrolled at the Colposcopy Clinic, IRCCS San Gerardo dei Tintori (Monza, Italy). All samples were sent and tested at the Laboratory of Clinical Microbiology of the University of Milano-Bicocca (Monza, Italy). Nucleic acids were extracted starting from 200 µl and hrHPV detection of 14 different genotypes was performed using the Anyplex™II HR HPV (Seegene) using Seegene Microlab NIMBUS.

Results: hrHPV positivity of 67% (235/351) was observed among enrolled women. Multiple hrHPV infections were observed in 57% (134/235) samples and HPV16 was the most common genotype detected. 127 women showed a positive colposcopy result and 79 required histological investigation. 72.2% (57/79) biopsies confirmed a clinically relevant lesion with the presence of a Cervical Intraepithelial Neoplasia (CIN) grade 2 or higher. A sensitivity of 96% and a specificity of 32% was observed. Some hrHPV genotypes were more frequently detected in women with CIN2+, such as hrHPV16, 18, 31, 45 and 58.

Discussion and Conclusions: Results have confirmed that the risk of high-grade cervical dysplasia varies strongly across hrHPV genotypes. This study contributes to the implementation of data regarding the use of HPV genotypes in the risk stratification of HPV screen-positive women. Infection

with hrHPV genotypes together with genotype-persistence are the main predictors of cervical cancer progression, underlining the importance of HPV genotyping in both HPV-primary screening and in the follow-up of HPV-positive women.

50 - ANALYSIS OF THE DISTRIBUTION OF GENOTYPES IN WOMEN REFERRED TO COLPOSCOPY WITH PERSISTENT HIGH-RISK HUMAN PAPILLOMA VIRUS (HPV) INFECTIONS

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Analysis of the distribution of genotypes in women referred to colposcopy with persistent high-risk Human Papilloma Virus (HPV) infections

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Introduction: High-risk HPV (HR-HPV) infections are frequently detected in sexually active women. Most of them are spontaneously cleared by women, while a small fraction can persist and cause cervical precancerous and cancerous lesions. Among HR-HPV genotypes, HPV16, HPV18 and HPV45 have been associated with a higher risk of developing cervical cancer. This ongoing study investigates the role of the different HR-HPV genotypes in the development of persistent infection in women referred to colposcopy.

Materials and Methods: 351 women referred to colposcopy were enrolled (T0) and followed-up according to the local clinical protocol at the Colposcopy Clinic of IRCSS San Gerardo dei Tintori (Monza, Italy) from May 2017 to April 2024. If required by the clinician, biopsy and/or conization treatment were performed. Presently, 127 women returned for the first follow-up (FU) visit, 43 for the second and 13 for the third. Cervical specimens were collected from women at each time point and tested with Anyplex™II HRHPV (Seegene) on the Microlab Nimbus platform to distinguish 14 HR-HPV genotypes. A persistent infection was defined as the presence of the same HR-HPV genotype at two consecutive time points.

Results: 67.0% (235/351), 48.0% (61/127), 34.9% (15/43) and 46.2% (6/13) of cervical samples collected during the first colposcopy visit and subsequent FU appointments were respectively HR-HPV-positive. HPV16 and HPV31 were the most frequently detected genotypes at all the time points. Conization was performed in 61 women at T0 and confirmed the presence of high-grade cervical lesions in 88.5% (54/61) of cases. HR-HPV persistent infections from one or more HR-HPV types at the first FU visit (6-12 months) were detected in 47 women for a total of 57 infections. In 19.1% of women (9/47) persistence was present in spite of previous treatment. Persistent infections in women not treated were associated with HPV31 (13/38 women), HPV16 (9/38 women), and HPV59 (5/38 women), while infections in women who underwent conization were mainly related to HPV16 (6/9 women). HR-HPV persistent infections at the second FU visit (12-24 months) were found in 11 women, for a total of

17 infections mainly due to HPV16 and HPV31. After 24 months no persistent infections from HPV18, HPV35, HPV39, HPV51 and HPV66 were detected.

Discussion and Conclusions: Different HR-HPV genotypes are not equally associated with persistence of infection and increased risk of cervical lesions following conization treatment. The introduction of HR-HPV genotyping as a triage test in HR-HPV-positive women may be a tool for the clinical management of women with cervical lesions.

73 - ACINETOBACTER BAUMANNII OUTBREAKS AMONG FOUR INTENSIVE CARE UNITS DEDICATED BOTH TO SARS-COV-2 POSITIVE AND NEGATIVE PATIENTS: DECIPHERING TRANSMISSION CHAINS

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Acinetobacter baumannii outbreaks among four intensive care units dedicated both to Sars-Cov-2 positive and negative patients: deciphering transmission chains

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Introduction

Multidrug-resistant (MDR) Acinetobacter baumannii is one of the leading causes of hospital-acquired infections. The SARS-CoV-2 pandemic saw a notable increase in MDR A. baumannii infections, that were among the most prevalent coinfections in COVID-19 patients in intensive care units (ICUs). Pathogenic microorganisms can spread in ICUs through direct contact between healthcare workers and patients, as well as contact with contaminated surfaces or medical devices.

Materials and Methods

From January 2020 to January 2022, four ICUs at Umberto I Teaching Hospital in Rome, underwent microbiological surveillance. Two ICUs were dedicated to COVID-19 positive patients (ICU-1C, ICU-2C), while the other two were for non-COVID-19 patients (ICU-1R, ICU-2R). Isolates relatedness was assessed using pulsed-field gel electrophoresis. Illumina whole genome sequencing was conducted on 26 representative isolates. Reads were assembled using Unicycler and annotated with Prokka. Single Nucleotide Polymorphisms (SNPs) were identified using Snippy. Roary and IQ-Tree were used for max-likelihood phylogenetic tree and Gubbins with BactDating for a SNP-based phylogenetic tree. Antimicrobial resistance (AMR) genes were identified using Comprehensive Antibiotic Resistance Database. Oxford and Pasteur Multi Locus Sequence Typing schemes, were obtained through Pathogenwatch.

Results

In total, 178 A. baumannii isolates were obtained from 129 COVID-19 patients and 49 non-COVID-19 patients admitted to the ICUs. Of these, 117 belong to the same clone (A). All the isolates were classified within the international clonal lineage II and exhibited a MDR phenotype. Carbapenems resistance (CR) and aminoglycosides resistance were primarily attributed to the presence of blaOXA-23 and armA genes, respectively. Outbreaks were detected between ICU-1R and ICU-R2, ICU-1C and ICU-R2, ICU-1C and ICU-C2, ICU-1C and ICU-R1, as well as within the wards themselves.

Discussion and Conclusions

Genomic and phylogenetic analyses allowed us to identify possible transmission routes of A. baumannii. All the isolates in our study were CR and the clone A was the only endemic one present in all wards. Other outbreaks derived from external introductions affected only a few patients, with exception of clone B that cause more than 30 cases in ICU-1C and ICU-1R2. Despite the measures taken during the COVID-19 pandemic, the observed outbreaks there was probably a decline in attention to normal care practices for the prevention of infections, which favoured the spread of MDR microorganisms among patients and between wards. Therefore, to address the problem effectively, it is essential to strengthen control measures and implement long-term strategies targeting MDR microorganisms even in ICUs stressed times.

89 - EPIDEMIOLOGICAL TREND OF ENTERIC PATHOGENS IN HOSPITALIZED PATIENTS: REAL-LIFE EXPERIENCE IN A CALABRIAN UNIVERSITY HOSPITAL

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ABSTRACT 52° Congress SIM 2024

Epidemiological trend of enteric pathogens in hospitalized patients: real-life experience in a Calabrian University Hospital

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Introduction: Acute Infectious Diarrhoea (AID) and associated short- and long-term complications are major causes of hospitalization worldwide. Patients with gastrointestinal infections can act as a potential reservoir of enteric pathogens, promoting outbreaks and failure of the effectiveness of public health interventions. In addition, enteric bacteria might continue to evolve during spread, through acquisition of antimicrobial resistance traits, as well as additional virulence factors. In Italy, limited data exist regarding their prevalence and circulation, due to the lack of robust surveillance programs. The aim of our study was to evaluate epidemiological trend of enteric pathogens among hospitalized patients with suspected AID attending to our teaching hospital. Materials and Methods: This cross-sectional retrospective study was conducted from January 2018 to December 2023. Stool samples were analyzed during routine diagnosis by conventional culture methods, syndromic molecular tests and immunochromatographic assays. Results: A total of 3261 stool samples were evaluated, with a percentage rate of 25% (805/3261) in 2018-2019, 17% (577/3261) in 2020-2021 and 58% (1879/3261) in 2022-2023. The most gastrointestinal infections were observed in 2018-2019 with 13% of stool samples resulted positive to at least one enteric agent, followed by 2020-2021 (8%) and 2022-2023 (7%). Fungal infection was the most prevalent (7-13%), mainly in oncological and critical ill patients, followed by Salmonella spp. (0.5-1%) and Campylobacter (0.3-0.5%). About Clostridioides difficile infections, a total of 2389 stool sample were analysed, of which 10% (234/2389) in 2018-2019, 27% (638/2389) in 2019-2020 and 63% (1517/2389) in 2022-2023. Among them, 9% resulted positive to glutamate dehydrogenase (GDH) antigen in 2018-2019, followed by 8% in 2020-2021 and 5% in 2022-2023. For all three-year periods considered, less than 1-4% of cases were due to C. difficile toxin A/B-producing strains. Regarding viral circulation, Norovirus GI/GII was the most prevalent (30/217, 14%), followed by Adenovirus F 40/41, Astrovirus, Sapovirus and Rotavirus (15/217, 7%). About parasite agents, Cryptosporidium (2%) and Giardia lamblia (1%) cases were observed. Overall, the major circulation of enteric pathogens appeared in gastroenterology unit, followed by oncology, infectious diseases and intensive care ward. Likewise, C. difficile was the most prevalent microorganism in

infectious diseases, intensive care and gastroenterology units. Discussion and Conclusions: Critically ill patients, such as oncological and individuals with chronic diseases, showed a greater proportion of enteric infections, particularly fungal and C. difficile ones. In 2020-2021 biennium were observed an increase of C. difficile toxin A/B-producing strains (4%), probably due to COVID-19 pandemic scenario. Our study emphasizes the importance of continuous surveillance on enteric pathogens at both local and global levels. Regular surveillance of prevalent enteric strains is useful in clinical practice to prescribe more appropriate interventions and to reduce an uncorrect use of antimicrobial agents.

90 - MOLECULAR EPIDEMIOLOGY OF GROUP A STREPTOCOCCUS ISOLATED FROM PATIENTS WITH SEVERE INVASIVE INFECTIONS IN ITALY DURING 2023-2024

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Molecular epidemiology of group A streptococcus isolated from patients with severe invasive infections in Italy during 2023-2024

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Introduction:

Severe invasive infections by group A streptococcus (GAS) have become a serious problem in various countries. Since September 2022, some European countries have reported an increase in the number of cases of invasive group A streptococcal disease (iGAS) among children under ten years and in adults over 65 years, some with fatal outcomes.

In Italy, except for scarlet fever, invasive GAS infection is not a notifiable disease, so it was not possible to verify whether, compared to previous years, there had been an increase in iGAS infections in our country too.

The working group dealing with beta hemolytic streptococcal infections in National Institute of Health (ISS) has received an increase in reports of iGAS cases and bacterial strains compared to only voluntary reporting from previous years. In particular, from December 2022 to April, 2024, 279 reports of iGAS were received, of which 266 were accompanied by the bacterial strain.

We presented the microbial investigation of GAS strains isolated from severe invasive diseases in Italy.

Materials and Methods: A total of 266 isolates were collected from patients with severe invasive GAS infections during 2023-2024 in Italy, and they were characterized by emm sequence typing. Species identification was confirmed by the Lancefield group. The presence of the superantigen speA and speC genes in all GAS, as well as tet, erm and mef resistance genes in all resistant isolates, was determined by PCR.
To distinguish between the recently identified toxicogenic M1UK variant and M1global strains, all emm1.0 isolates received were analyzed by allele-specific PCR in rofA gene using SNP and WT primers.

Results

In the period September 2022 – April 2024, the ISS received over 260 strains. Among them, emm1 (48%) was the dominant serotype, followed by emm 12 (14%), both in the general population and distinguishing between the paediatric age group (0-19 years old) and adults. For the first time on April, 2024, ISS identified 3 strains from adults affected by iGAS infection caused by the emm3.93

serotype. Patients were from two different regions. All the emm1 isolates had the gene for the superantigen speA, and approximately 50% of the emm1 isolates presented the toxicogenic M1UK variant.

Conclusions

This study confirms changes in the epidemiology of GAS disease in Italy, such as the presence of emm-type 3,93 strains and the presence of toxicogenic M1UK variant. Clinical studies are required to assess whether invasive group A streptococcal infections caused by the M1UK variant are more severe. Continued invasive disease surveillance and characterization of iGAS should be done for assessing the accurate streptococcal invasive disease burden in our population.

95 - VARICELLA ZOSTER VIRUS ANTIBODY TITER IS NOT A GOOD MARKER OF THE CLINICAL PROTECTION DRIVEN BY CHICKENPOX VACCINE

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Title: Varicella zoster virus antibody titer is not a good marker of the clinical protection driven by chickenpox vaccine

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Background: The introduction of mandatory Varicella-Zoster Virus (VZV) vaccination during childhood, integrated as a compulsory measure in Italy in the National Vaccine Prevention Plan (PNPV) in 2017, has substantially reduced the overall incidence, complications and childhood hospitalizations associated with this infection. Our aim was to investigate the possible impact of vaccination on the overall immune response to VZV infection by comparing seroprevalence and antibody levels in pre- and post-vaccination era.

Materials: A retrospective analysis was carried out on 10,921 serum samples from patients that underwent screening and quantification of VZV-specific IgG at Bambino Gesù Children Hospital from January 2013 to December 2022, by using the chemiluminescence immunoassay (CLIA) technology.

Results: Overall, we observed a VZV seroprevalence of 79.7% in our general population. Median age was 19.3 years (IQR: 7.7-32.7). Individuals were classified into 9 age groups (Table 1). Seroprevalence showed an increasing trend starting in the younger age groups: <1 year, 49,4%; 1-5 years, 53,4%; 6-9 years, 68,3%; 10-14 years, 78,1%; 15-19 years, 86,9%; 20-39 years, 91,4%; 40-64 years, 94,1; 65-71 years, 98,4; >74 years, 96,0%.

Among the overall serum, 2,445 were collected in the pre-vaccination era (2013-2016) and 8,476 in the post-vaccination era (2017-2022). Comparing VZV antibodies prevalence in the different age groups, no significant difference was found between the two eras. However, a notable decrease in antibody titers was observed in post-vaccination era, particularly among age groups <10 years (Table 2). Moreover, we observed an 87% reduction in the median number of hospitalizations after vaccination era.

Conclusion: These results show a reduction in antibody titres in the post-vaccination era compared to the pre-vaccination era. These findings, taken in isolation, may initially raise doubts about the efficacy of the vaccine. However, a plausible explanation is that vaccine-induced immunity leads to the production of antibodies that are not easily detectable by standard VZV tests used in clinical practice. Furthermore, these tests do not distinguish antibodies produced by natural infection from those

induced by vaccination. Consequently, relying on conventional methods to measure VZV-specific Ig may not be the optimal choice for assessing the impact of vaccination.

Table 1. Study population

Total, N	10.921
Serum in the pre-vaccine era, N (%)	2445 (22,4)
Serum in the post-vaccine era, N (%)	8476 (77,6)
<1 year ,N (%)	318 (2,9)
1-5 years ,N (%)	1857 (17)
6-9 years ,N (%)	1111 (10,2)
10-14 years ,N (%)	1352 (12,4)
15-19 years ,N (%)	893 (8,2)
20-39 years ,N (%)	4068 (37,2)
40-64 years ,N (%)	1146 (10,5)
65-74 years ,N (%)	126 (1,2)
>74 years ,N (%)	50 (0,5)

Table 2: The median VZV-specific IgG titre levels (IU/mL) for each age group in the pre- and post-vaccination era. The last column shows the p-value data comparing both eras within each age group.

	2013-2016 Era			2017-2022 Era		
Age (year)	Median Title IgG (UI/mL)	IQR	Age (year)	Median Title IgG (UI/mL)	IQR	p
<1 y (N=39)	688.9	388.4-1218.0	<1 y (N=253)	356.9	173.8-755.6	0.003
1-5 y (N=174)	1086.5	397.6-1950.0	1-5 y (N=1,471)	595.1	275.4-1394.0	<0.001
6-9 y (N=131)	1305.0	556.7-2177.0	6-9 y (N=917)	949.2	458.8-1874.5	0.038
0-14 y (N=156)	1245.0	513.7-1963.5	10-14 y (N=1,144)	1063.5	563.1-1811.0	0.702
5-19 y (N=103)	1414.0	717.3-2068.0	15-19 y (N=772)	1106.0	600.9-1942.0	0.281
0-39 y (N=980)	1453.0	736.2-2032.0	20-39 y (N=2,977)	1136.0	669.5-1773.0	<0.001
0-64 y (N=310)	1524.0	780.1-2078.0	40-64 y (N=808)	1217.5	696.7-1915.0	0.008
65-74 y (N=32)	1352.0	813.9-2003.5	65-74 y (N=93)	1272.0	784.9-1868.0	0.732
>74 y (N=9)	1536.0	1075.0-2275.0	>74 y (N=8,476)	1459.0	772.7-2453.0	0.567

126 - TRACKING THE EVOLUTION OF CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE: A NINE-YEAR GENOMIC STUDY IN SAN MATTEO HOSPITAL, PAVIA

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Tracking the evolution of Carbapenem-resistant Klebsiella pneumoniae: a nine-year genomic study in San Matteo hospital, Pavia

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Introduction. Carbapenem-resistant Klebsiella pneumoniae (CRKp) represents a serious challenge in healthcare settings, with limited treatment options and high mortality. CRKp isolates usually harbor genes encoding for carbapenem-degrading beta-lactamases, such as KPC and NDM. These genes are conveyed by plasmids that are easily spread among high-risk global clones, including clonal group (CG) 258 and the emerging CG307. Given the role of CRKp in nosocomial infections, our study investigates its epidemiology through genomic surveillance at San Matteo Hospital (HSM; Pavia, Northern Italy) over a nine-year period.

Materials and Methods. From January 2015, to September 2023, we selected 556 phenotypically-confirmed CRKp isolates, representing at least 20% of the total CRKp isolates in each hospital area and year quarter at HSM. These included previously sequenced genomes from past projects and new sequences obtained ad hoc. We analyzed sequence types (STs), resistome, and virulome using Kleborate. For the most prevalent STs, a core-SNPs maximum-likelihood phylogeny was inferred.

Additionally, plasmid content was evaluated using Deeplasmid and an in-house approach that combines openTSNE for dimensionality reduction and HDBSCAN for clustering, enabling us to trace plasmid content clusters (PCCs).

Results. Over nine years, 37.05% of CRKp isolates at HSM belonged to CG258 (142 to ST512 and 64 to ST258), 32.49% to ST307, 9.03% to ST6668, and 5.78% to ST101, with the remaining distributed among various less common STs. Predominantly, CG258, ST307, and ST101 exhibited KPC-2 and -3, while ST6668 harbored NDM-1. Phylogenetic analyses identified 81 clusters: 39.02% were single-genome events, 34.15% were low-transmission clusters (2-4 genomes), and 25.61% were epidemic clusters (ECs; 5+ genomes). The dynamics of EC spread varied for each ST. Some ECs have persisted at HSM since 2015, while others have declined (e.g. of both cases in ECs of CG258). Moreover, new clusters emerged lately, especially within ST307. We identified 19 main PCCs, typically ST-associated. We observed considerable variability within the STs, especially among those persisting over the years. Notably, the same PCCs were not only shared among multiple ECs of the same ST, but also across different STs, as between ST307 and ST392 (CG147).

Discussion and conclusions. Phylogenetic analyses revealed multiple CRKp introductions and clone circulation, with occasionally local ECs expansion in HSM among years. Plasmid content sharing within the same ECs was expected. Nevertheless, the diversity in PCCs despite strain's high clonality, as in ST6668, suggests possible inter-EC transfer, even extending beyond STs. Plasmid transfer patterns could help understand resistance and virulence dissemination, crucial for enhancing global surveillance and, in turn, infection management.

127 - FOGS: A NOVEL TIME-AWARE INDEX OF BACTERIAL GENOME PLASTICITY TO PREDICT EMERGING HIGH-RISK CLONES

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FOGS: a novel time-aware index of bacterial genome plasticity to predict emerging high-risk clones

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Introduction. For years, clinical bacteriology has been dealing with hopeful monsters, i.e. strains that rapidly change their gene content, potentially becoming dominant in hospital settings. These are often associated with multidrug-resistance and hypervirulence. Among what drives the rise of hopeful monsters is the rate at which bacterial strains are able to change their gene content, which can be defined as Plasticity. Previously, gene content variation has been measured with indices based on presence/absence of orthologous genes and calculated as the mean value of all pairwise distances among the genomes in analysis (e.g. in 10.1186/s12862-018-1261-7, 10.1371/journal.pgen.1008114, and 10.1186/1471-2164-12-32).

Materials and methods. Available indices do not take into account that genes can move together and, most importantly, they measure pairwise distances in terms of gene content, without considering the rate at which such content changes. We conceived a new index named Flux of Gene Segments (FOGS). Pairwise distances are calculated as the number of DNA exchange events, regardless of their size (computed using adjacency graphs, similarly to 10.1186/1471-2164-14-309), divided by the distance in coreSNPs (obtained with 10.1093/bioinformatics/btad571), used as a proxy of evolutionary distance. To benchmark our method, we divided three collections of high-quality genomes of Klebsiella pneumoniae, Escherichia coli, and Staphylococcus aureus into subpopulations (using 10.1093/nar/gkz361) and measured their plasticity using FOGS.

Results. In all three analyzed bacterial species, clusters with higher FOGS correspond to global emerging high-risk clones (identified with STs). In K. pneumoniae, ST307 and ST101 are the most

plastic, while the declining ST258 has less than half of the FOGS value of ST307. Moreover, the most plastic clusters are associated with higher resistance scores. In S. aureus and E. coli, prominent STs are split into two clusters, with only one of them having high FOGS values (most of their dataset). S. aureus high-plasticity cluster ST8A is associated with methicillin-resistance gene mecA, opposite to cluster ST8B.

Discussion and conclusions. FOGS is based on the rate of gene content variation and can be used to measure plasticity. Moreover, it may be used to recognize and predict emerging strains of clinical importance, even at the subclone/subST level.

134 - AN OUTBREAK OF PARVOVIRUS B19 IN ROMAGNA AREA

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an outbreak of parvovirus b19 in romagna area

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Introduction: Human parvovirus B19 (B19V) is a ssDNA virus of the Parvoviridae family, it has a wide clinical spectrum, ranging from an asymptomatic infection to a life threatening one. During pregnancy, it can lead to fetal loss and hydrops fetalis. It affects children in the school environment, at the beginning of the spring, but can be contracted at any time of the year. Epidemic peaks typically occur every 3-4 years. In children, school aged, B19V classically causes erythema infectiosum (fifth disease), a bi-phasic fever and rash illness. Erythema infectiosum is characterized by a prodromal phase attributed to B19V viremia followed by the classical “slapped cheek” rash, an erythematous exanthem with circumferential pallor, corresponding to immune activation. The rash can later spread to the trunk and limbs, often as an erythematous reticular-type rash. Transient Aplastic Crisis, a temporary suspension of red-blood cell production, is potentially a more serious manifestation of B19V, with severe anemia and related complications. The appearance of the facial rash is usually enough to diagnose B19V infection, confirmation can be made through the assay of specific immunoglobulins of IgG and IgM classes or, especially for people at risk, from the search for viral DNA. Material and Methods: This document has been prepared to ascertain the seroprevalence of B19V infection in Romagna area (Italy). For the quantitative determination of specific IgG and IgM antibodies to Human B19V in human serum samples a CLIA was used assay by LIAISON®. The data considered are those determined from January to April 2023 and 2024 in comparison, divided into age groups, area of origin and gender. Positive results for IgM, likely classified as acute infection, were also compared for the B19V DNA test. Results: The results show a higher positivity rate in 2024 compared to the same period last year. There is a greater number of women of childbearing potential if we consider female subjects. The DNA test confirmed a high viral load with viremia values >104 copies/mL, confirming acute infection. Discussion and Conclusions: Every epidemic cycle makes this virus the protagonist of physical discomfort with consequences that can be very serious during pregnancy or immonudepression. In addition, the higher incidence in the school-age group favors transmission to women (mothers). This work aims to address the demand to test for B19V during pregnancy as is the case for other viruses. For some of these, in fact, there is a high risk of leading to malformation of the fetus, and even if B19V infection does not have the same consequences, it can have dramatic outcomes for a woman because an early infection could lead to miscarriage or hydrops fetalis.

140 - VANCOMYCIN-RESISTANT ENTEROCOCCI IN IMMUNOCOMPROMISED HOSTS: A GENOMIC ANALYSIS

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Vancomycin-resistant enterococci in immunocompromised hosts: a genomic analysis

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Introduction. Vancomycin-resistant enterococci (VRE) represent a severe nosocomial concern. VRE colonization and infection correlate to several risk factors, such as previous antimicrobial therapy, prolonged recoveries, and inadequate patient management. In addition, precise sequence types (ST) correlate to specific virulence factors and resistance markers, which help the microorganisms' diffusion within healthcare settings and fragile hosts. We recently observed a VRE increase in immunocompromised patients, and addressed the problem using a multifaceted approach. Herein, we describe the genomic results on the haematological patients' VRE isolates, isolated during 2023.

Materials and methods. The study screened 18 Enterococcus spp. strains isolated from the University Hospital Policlinico of Catania haematology unit. Samples such as urine, blood, stool, and drainage fluids were processed through culture exams, and Enterococcus spp. isolates underwent a VRE confirmation through phenotypical and molecular assays. A whole genome sequencing (WGS) defined strains STs, resistome, and virulome.

Results The study identified 13 Enterococcus faecium and 5 Enterococcus faecalis, showing glycopeptides resistance due to a vanA carriage. The most identified STs (E. faecium ST80, ST117, and E. faecalis ST28) matched the European epidemiology. E. faecium ST80 and ST117 belong to Clonal Complex (CC) 17, containing hospital-clade-specific genes, including resistance genes and a pathogenicity island (esp genes). Although the ST28 prevalence, an ST179 E. faecalis revealed supplementary adhesion and biofilm formation markers. According to this evidence, the ST179 strain originated from a urine sample of a catheter-implanted patient. The virulome analysis highlighted differences in adherence capability for E. faecalis compared to E. faecium.

Discussion. Generally, the observation of the VRE increase in immunocompromised patients can be explained by different aspects, including strain-specific markers and comorbidities of these patients. Notably, most patients underwent antineoplastic chemotherapy and third-generation cephalosporins prophylaxes, possible risk factors for enterococcal proliferation and resistance development. Further studies should investigate potential host-pathogen interactions to uncover new virulence mechanisms or patients' clinical factors eventually related to VRE increased incidence among immunocompromised hosts.

148 - OVERVIEW OF ADENOVIRUS RESPIRATORY INFECTIONS: PRELIMINARY FINDINGS OF A MULTICENTRE STUDY IN ITALY, 2022-2023

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Overview of Adenovirus respiratory infections: preliminary findings of a multicentre study in Italy, 2022-2023

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Introduction. Human Adenovirus (hAdVs) typically causes mild respiratory infections, but it could lead to severe syndromes, particularly in children or immunocompromised individuals. The present study was promoted by the Working Group on Respiratory Virus Infections (GLIViRe) of the Italian Association of Clinical Microbiologists (AMCLI). The study objectives are to investigate hAdV respiratory infections among adults and children and assess the prevalence of different hAdV genotypes during the study period.

Materials and Methods. This study included 34,112 respiratory samples collected from eleven Italian clinical laboratories between January 2022 and June 2023. Samples were screened to detect hAdV-DNA and a specific nested PCR targeting the hexon gene was performed on a part of hAdV-positive samples to determine their genotype.

Results. A total of 1,772/34,112 (5.2%) were hAdV-positive. The majority of hAdV-positive samples were detected from March 2023 through the end of April, with a positivity rate of 13.4% (Fig.1). Among the hAdV-positive cases there were 988 males and 773 females (55.7% and 44.3%, respectively), with a median age of 3 years (range: 24 days-98 years) (Fig.2). In 867/1772 cases (75.7%) at least one other respiratory virus was detected, alongside the hAdV. Among these cases, Rhinovirus was the most commonly detected (449/867, 51.8%) followed by Respiratory Syncytial Virus (80/867, 9.2%) and Metapneumovirus (65/867, 7,5%). hAdV typing was performed on 158/1772 (8.9%) collected samples between July 2022 and June 2023. The most common strain was hAdV-B3 identified in 62% of cases (98/158), followed by hAdV species C strains, including hAdV-C2 (27/158, 17%), hAdV-C1 (19/158, 12%), and hAdV-C5 (7/158, 4.4%).

During the study period, a shift in circulating genotypes has been observed. From July 2022 to January 2023, hAdV-C strains were the most commonly detected. From February 2023 until the conclusion of the study period, hAdV-B3 was the prevailing strain circulating in Italy (Fig.3).

Discussion and conclusion. hAdV accounts for 5% of all respiratory infections and children are particularly susceptible to hAdV respiratory infections. hAdV was present throughout the study period, with an increase in cases during the spring of 2023, sustained by the hAdV-B3 strain.

159 - DETECTION OF CLOSTRIDIOIDES DIFFICILE TOXINS IN RECTAL SWABS OF PATIENTS AT EMERGENCY DEPARTMENT: A RETROSPECTIVE, PRELIMINARY SINGLE-CENTER STUDY

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DETECTION OF Clostridioides difficile TOXINS IN RECTAL SWABS OF PATIENTS AT EMERGENCY DEPARTMENT: A RETROSPECTIVE, PRELIMINARY SINGLE-CENTER STUDY

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DETECTION OF Clostridioides difficile TOXINS IN RECTAL SWABS OF PATIENTS AT EMERGENCY DEPARTMENT: A RETROSPECTIVE, PRELIMINARY SINGLE-CENTER STUDY

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Introduction

Clostridioides difficile is responsible for 20-30% of cases of antibiotic-associated diarrhea and is the main cause of infectious diarrhea in the hospital setting, and its incidence is constantly increasing.

C. difficile is found in the feces of 3% of healthy subjects and the highest colonization rate is found in healthy children in the first year of life, but the prevalence rises to 20-50% in hospitalized patients.

In case of infection the patient can mainly suffer colitis. The clinical pictures are determined by the action of the exotoxins A and B, acting individually or together, produced by the bacterium. The more severe pseudomembranous colitis is related with the ability to produce the binary toxin (CDT), not related with A and B toxins.

Considering the potential lethality of the infection, an early clinical and laboratory diagnosis is essential. The latter is mainly based on isolation and analysis by ELISA of toxins (A, B and binary) on rectal swabs or in feces, as well as on RT-PCR for the detection of the genes accounting for their production, and especially those for binary toxin produced by the hypervirulent E27 strains carrying the tcdC-117 deletion.

Materials and Methods

We retrospectively evaluated the incidence of positive rectal swabs performed in 1500 patients admitted at Emergency Department at Policlinico of Bari during the period January 2019 - December 2020. To search the genes tcdB, ctdA and tcdC, encoding toxin B, CDT subunit A, and the negative regulator of toxins A and B, the samples were pretreated for DNA extraction and identification with XPERT® C. difficile assay (Danaher, Washington D.C., US). The test was performed by RT-PCR using the Gene-XPERT® system (Danaher, Washington D.C., US) Furthermore, the assay is able to identify the tcdC-117.

Results

A total of 1500 rectal swabs were analyzed for the detection of C. difficile toxin, 748 (49.9%) from female subjects (mean age 63.1 years) and 752 (50.1%) of male subjects (mean age 62 ,8 years).

Out of a total of 1500 tests, there were 173 (11.5%) positive for the detection of toxin B, 53 (3.5% of the total) subjects were positive for the detection of binary toxin, and 35 (2.3%) were positive for tcdC-117 deletion. In a few cases, both toxin A and B were positive in the same sample.

Discussion and Conclusions

In conclusion, the screening for C. difficile performed at admission at Emergency Department revealed several patients with a potentially severe infection. In particular, the higher incidence was recorded for strains producing B toxin, and a not negligible proportion of carriers of hypervirulent strains. This high incidence must be considered to adopt early protocols to prevent infectious complications and negative outcomes.

167 - MOLECULAR EPIDEMIOLOGY OF RESPIRATORY VIRUSES IN AUTUMN-WINTER 2023-24 IN ROME

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Molecular epidemiology of respiratory viruses in autumn-winter 2023-24 in Rome.

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Introduction. After a nearly complete absence of respiratory viruses other than SARS-CoV-2 cases during 2020-21 winter season due to pandemic restrictions, a resurgence of respiratory syncytial virus (RSV) cases in autumn 2021 and an intense circulation of RSV and Influenza viruses (Flu) in the epidemic season 2022-23 were reported worldwide. The aim of this study was to monitor respiratory viruses in the 2023-24 epidemic season in children and adults.

Material and Methods. Children older than one year and adults consecutively attending the Sapienza University Hospital of Rome for acute respiratory infections from October 1, 2023 to March 31, 2024, were tested for respiratory viruses by molecular methods in nasopharyngeal swabs.

Results. A total of 238 children (1-17 years) and 508 adults (18-94 years) were tested for respiratory viruses. The most common virus detected in all age groups was FluA: 65/238 children (27.3%) and 171/508 adults (33.7%). FluB was the second most common virus in children (39/238: 16.4%), but was detected in only about 1% of cases in adults. As expected, RSV-positive cases were more common in children (38/238: 16%) than in adults (31/508: 10.4%), with RSV-A dominating over RSV-B. Rhinoviruses accounted for about 14% of the total cases, equally distributed among all age groups, while the other respiratory viruses were more frequent in children. Hospital admissions increased from the second half of November 2023 with a peak at the beginning of January 2024, but continued until March.

Discussion and Conclusions. The seasonal peak of influenza hospitalizations in 2023-24 occurred earlier and was more intense than in the post-pandemic years, probably due to residual immune debt

in the population. Interestingly, RSV circulation appeared to be less intense but more prolonged than in the previous two seasons. In contrast, FluB and HRV circulated abundantly in early spring. Molecular diagnosis of respiratory infections can help rationalize health care resources, monitor the onset and intensity of seasonal peaks, effectively administer influenza antivirals, plan passive and active RSV prophylaxis, and build resilience against future pandemic threats.

184 - COMPARISON BETWEEN CHILDREN AND ELDERLY SARS-COV-2 INFECTED POPULATIONS. FOOTPRINT OF DIFFERENT GENOMIC MUTATIONS THAT COULD DRIVE VIRAL FITNESS AND EVOLUTION.

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SIM 8-11 SETTEMBRE 2024 PAVIA

TOPIC: 1) Epidemiologia

Comparison between children and elderly SARS-CoV-2 infected populations. Footprint of different genomic mutations that could drive viral fitness and evolution.

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Introduction

Application of next-generation sequencing (NGS) as an innovative tool for epidemiological surveillance during COVID-19 pandemic has showed its valuable role to monitor and detect viral genome variations. Worldwide data have been collected over the last 3 years to follow different SARS-CoV-2 variants. However, little has been described to understand different trend between children and elderly infected populations, such as typical mutations related to less or severe manifestations.

Investigations into these aspects could be relevant to dissecting epidemiological signatures to anticipate viral behavior.

Material and Methods

1455 positive nasopharingeal swabs, from children (<14 years) and elderly people (>65 years) were extracted and amplified with automated harmonized methods between Verona and Mestre Hospitals. Samples with Ct<30 were arranged in separate libraries and sequenced with NGS technology. Analyses were performed with custom and commercial bioinformatic pipelines. Data obtained were put together.

Results

We found significant differences in lineages frequencies and mutations between two age groups during the last three years' pandemic. BA.2*(17,99%), BA.5.2* (14,39%), and BQ.1* (10,07%) Omicron sublineages were commonly detected in children, instead of BQ.1* (15,20%), XBB* (11,55%), and BA.2*(9,65%) Omicron sublineages in elderly. Regarding mutations, children showed a specific pattern composed by higher variations on Orf1ab and S genes. In particular, aside from the S:A27S Omicron mutation, we found that S:Q146H (77,7%) and ORF1a:R47K (76,98%) mutations are specifically present in this group. Considering elderly, S:E183Q (66,72%) and S:A83V (66,03%) mutations were more frequent than young people. ORF1b:P959S was found in both groups (79.87% in children vs 68.09% in elders).

Discussion and conclusions

Considering data in their complexity, the attempt to segregate SARS-CoV-2 sequencing by age groups provides a more in depth analysis of viral dynamic, such as exploiting replication in children reservoirs and maintenance in elderly. This hypotesis is supported by higher mutation findings in replicase genes (Orf1ab) amoung young people, of which many were not vaccinated. Conformational changes in spike genes, on the other hand, might have strengthened SARS-CoV-2 circulation in older people, probably with comorbidities. Unreavealing these differences could help to identify which subgroup can be used by the virus as a prototype for its evolution over time. Results of this study could also help to make early prevision on viral changes to comprehend how viral mutational rate could drive a more severe disease in elderly population.

191 - HTLV-1: A NEGLECTED VIRUS IN ITALY

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HTLV-1: a neglected virus in Italy

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Introduction. The human T-cell leukemia virus type 1 (HTLV-1), is highly spread in endemic regions in the world such as Southwestern part of Japan, sub-Saharan Africa and South America, Caribbean, Middle East and Australo-Melanesia regions. The routes of transmission of HTLV-1 are mother-to-child, mainly through breastfeeding, transfusion of non-leucocyte depleted blood, shearing needles in intravenous drug users, and sexual intercourse. Primary HTLV-1 infection is silent and remains so in most infected individuals. However, after years of latency, HTLV-1 can cause adult T cell leukemia (ATL) or inflammatory conditions, most notably HTLV-1-associated myelopathy/tropic spastic paraparesis (HAM/TSP), in 5-15 % of all infected persons. ATL and HAM/TSP are incurable at the moment. An estimate of individuals living with HTLV-1 worldwide, in 1993, was around 10-20 millions. Due to the lack of global population-based prevalence studies, this is considered an underestimate at the moment. Furthermore, HTLV-1 prevalence is impacted by migration. Particularly, no data on HTLV-1 prevalence in the general population in Italy are available. Methods. We carried out a systematic literature review of studies conducted in Italy on HTLV-1/2 from 1980 to 2023, using the free database PubMed and the words “HTLV” and “Italy” as search criteria. Moreover, we made a theoretical estimation of foreign individuals living with HTLV-1 in Italy, based on numbers of foreigners by country of citizenship officially registered as resident in Italy on 1st January 2024 (ISTAT) and data on % prevalence of HTLV-1 in the general population of different countries in the world, mainly derived from the “Human T-Lymphotropic Virus Type 1: Technical Report, WHO, 2021”. Results. Based on the criteria we adopted, a total of 426 publications were found (64 reviews, 99 epidemiological and 263 translational studies). Selected publications concerned specific local realities, isolated case reports, clinical studies on a very limited number of individuals, or preclinical studies. None addressed the general situation of HTLV-1/2 infection in Italy. The results of the arithmetical estimation indicate that around 26000 individuals are living with HTLV-1 in Italy, only among officially registered foreigners from highly endemic areas. Conclusions. Based on our study we can deduce that HTLV-1 is a neglected

virus in Italy and that HTLV-1 associated diseases are rarely reported or misdiagnosed in our country. We think that now the time has come to change the present state of things and to start, similarly to what has been done in other European countries, with a serious surveillance on HTLV-1/2 in Italy, beginning from a centralized, mandatory reporting of all HTLV-1/2 performed tests.

193 - THE GROWING TREND OF SEXUALLY TRANSMITTED INFECTIONS IN YOUNG PEOPLE IN A NORTH-EAST ITALY AREA FROM 2017 TO 2022.

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The growing trend of Sexually Transmitted Infections in young people in a North-East Italy area from 2017 to 2022.

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Introduction: Recent European data documented the increase of Sexually Transmitted Infections (STIs) notification with particular attention to N. gonorrhoeae, among heterosexual young people, surpassing the number of reported cases before the COVID-19 pandemic. The Italian epidemiological report (2020-2021) highlighted an increase of STIs prevalence in the at-risk population (18%), although no additional case of N. gonorrhoeae was reported in younger population. In Italy, the local health restrictions adopted during the COVID-19 pandemic could have differently influenced the clinical management and data transmission of positive cases. To assess the impact of the COVID-19 pandemic on the spread of STIs within the Italian population, we analyzed microbiological data from both pre-and COVID-19 periods. This analysis was conducted in a geographic area where only minimal limitations were imposed on STD clinical and laboratory services. Materials and Methods: This retrospective study (from 2017 to 2022) included 5503 subjects, 2586 STI clinic attendees (STD-group) and 3687 patients with a diagnosis of primary infertility (ART-group). The samples were tested for M. hominis/genitalium, U. urealyticum/parvum, C. trachomatis, N. gonorrhoeae and T. vaginalis by a multiplex PCR. Additionally, N. gonorrhoeae positive samples were tested for ciprofloxacin and azithromycin resistance genes. Results: In this area, the overall STIs prevalence during COVID-19 pandemic showed a significant increase compared to the previous period ($p<0.01$). In STD-group, U. parvum represented the most frequent microorganism detected (26.1% vs 23.9%) with a significant increase rate in women (52.1% vs 32.7%) ($p<0.001$), and with a frequency as multiple infection twice higher than the pre-COVID-19 period ($p<0.01$). A significant decrease in positive rates was observed for

C. trachomatis (12.1% vs 18.8%) ($p<0.001$), and for M. hominis (11.4% vs 16.4%) ($p<0.01$) during pandemic. No relevant change was observed for T. vaginalis (0.9% vs 2.8%). A constant increase of N. gonorrhoeae was observed in young people (aged 19-29) where more than half of the positive subjects reported to be heterosexual. A high spread of N. gonorrhoeae resistance to ciprofloxacin was detected in this age group, likewise in people over 40 years old. In ART-group U. parvum was the most frequently detected microorganism found as single infection. This bacterium was largely detected in infertile young women (19y-29y, $p=0.01$) together with M. hominis ($p=0.01$). Discussion and Conclusions: This retrospective study based on laboratory data from 2017 to 2022 showed an increase of notifiable STIs during COVID-19 in young subjects including heterosexual. The magnitude of this trend will have to be evaluated in the coming years.

196 - FIVE YEARS OF GENOMIC SURVEILLANCE OF CANDIDA ISOLATES FROM BLOOD INFECTIONS IN A LARGE HOSPITAL

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Five years of genomic surveillance of Candida isolates from blood infections in a large hospital

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Introduction

Candida are yeasts that typically exist as commensal, residing on the mucosal surfaces and within the respiratory or gastrointestinal tracts. Under conditions of host vulnerability, Candida species can act as opportunistic pathogens, resulting in diverse forms of invasive candidiasis, including candidemia. Genomics of pathogenic yeasts can shed light on their epidemiology, evolution and molecular mechanisms of pathogenesis and resistance to antifungal compounds.

Materials and methods

The dataset used in this study comprises 111 samples of Candida spp isolated from patients with candidemia from hospital San Matteo in Pavia during a span of 5 years, from 2015 to the first COVID wave in spring 2020. Antifungal susceptibility testing was performed and Minimum inhibitory concentrations (MICs) were assessed. Genomes of all isolates were sequenced with Illumina sequencing. Sequencing reads from isolates belonging to C. albicans and C. parapsilosis, representing the majority of the dataset, were mapped to the corresponding reference genomes. Single Nucleotide Polymorphisms (SNPs) were determined and used to reconstruct species phylogenies and epidemiological connections. Genetic variant annotation and functional effect prediction was performed.

Results

Our dataset shows an increase in C. parapsilosis (81) and C. albicans (18) cases observed from 2018 to 2020. Focusing on the most abundant C. parapsilosis, we determined that 61 isolates result to be highly similar at the genomic level, with distances below 35 SNPs from each other, thus belonging to a strain capable of persisting in the hospital. All the isolates belonging to the outbreak are resistant to fluconazole. Regarding the other azoles, itraconazole and voriconazole, we observed more diverse resistance profiles. The 20 C. parapsilosis isolates not belonging to the persistent strain resulted to be susceptible to all three antifungals.

All the isolates of the persistent strain present the most common mutation for fluconazole resistance in the ERG11 gene (Y132F). Interestingly, all these isolates, with the exception of the single isolate susceptible to both itraconazole and voriconazole, also present one missense mutation in MRR1 gene. This gene is a transcription regulator of CDR1 and MDR1 (genes encoding for efflux pumps).

Discussion and conclusion

Our study describes the largest Candida genomic dataset from Italy to date and highlights the potential of genomics to trace the epidemiology of fungal pathogens, also allowing to determine association between genotype and resistant phenotypes.

205 - MICROORGANISMS INVESTIGATION IN UNTREATED AND TREATED WASTEWATER SAMPLES OF CAMPANIA REGION

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Microorganisms investigation in untreated and treated wastewater samples of Campania Region

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Introduction

Wastewater contain biological products originated by the population, including bacteria and viruses. In wastewater treatment plants (WWTPs), different steps of treatments are conducted on sewage to eliminate contaminants in them, and bacteria and viruses are inactivated by chlorine and its derivatives. The aim of this study was to evaluate the efficacy of treatments in reducing bacterial load in two WWTPs located in Campania region.

Materials and Methods

From May 2023 to April 2024, 36 wastewater samples were collected in two WWTPs (named WWTP 1 and WWTP 2) once a month (with few exceptions). In particular, 21 originated in WWTP 1 from May 2023 to March 2024, and 15 samples in WWTP 2 from July 2023 to March 2024. For every sampling there was a raw sample, a pre-chlorinated sample (both collected as a 24h composite sample) and a post-chlorinated sample (collected as grab sample). The depurative process is chemical-physical for WWTP 1 and biological-active sludge for WWTP 2. The investigated bacteria were total coliforms, intestinal enterococci and Escherichia coli, whose identification was conducted through membrane filtration following the ISO (International Organization for Standardization) norms: UNI EN ISO 9308-1:2017, UNI EN ISO 7899-2:2003 and APAC CNR IRSA 7030 F Man 29 2003, respectively.

Results

In all samplings, bacterial load of total coliforms and E. coli varied in the range of 106 and 107 CFU (colony-forming units)/100 mL, and in the range of 105 and 106 CFU/100 mL for enterococci. From the raw to the pre-chlorinated sample, it was observed a reduction in bacterial load of at least an order of magnitude, and a reduction in bacterial load of another order of magnitude for all the tested microorganisms passing from the raw to the post-chlorinated sample, with few exceptions, in which an increase of bacterial load was observed. Several factors can influence this result, such as the

volume chosen for the analysis. Bacteria were not detected in the post-chlorinated sample only in WWTP 1 in May 2023, for E. coli and enterococci.

Discussion and Conclusions

This study confirmed that wastewater treatments are efficient in reducing bacterial load in sewage, but not in eliminating it. This suggests that treated wastewater can be used for other purposes since its bacterial load is reduced, but poses the need to implement new treatments for a better and complete removal of bacteria from sewage. Further studies will have to be conducted to confirm these results, and compare the situation in other WWTPs.

216 - NINE-YEAR EPIDEMIOLOGY OF GROUP A STREPTOCOCCI AND WHOLE GENOME SEQUENCING OF INVASIVE STRAINS ISOLATED IN AN ITALIAN HOSPITAL

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Nine-year epidemiology of group A streptococci and whole genome sequencing of invasive strains isolated in an Italian hospital

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Introduction

Group A streptococci (GAS) causes pharyngitis, superficial skin infections, pneumonia, meningitis, bacteraemia, cellulitis, streptococcal toxic shock syndrome, and necrotizing fasciitis, with a large burden to health-care systems. In 2022 five member States of European Union reported an increase in the number of paediatric invasive GAS (iGAS) infections. The aim of this study is to investigate the epidemiology of GAS infections from 2015 to July 2023 among the patients of the Fondazione IRCCS Policlinico San Matteo (Pavia, Italy), a 900-bed hospital.

Material and Methods

The evaluation of the distribution of isolates was done considering age of the patient, types of samples, and date of sampling. GAS strains isolated during 2023 from invasive infections were sequenced through Illumina MiSeq and analysed by manually searching virulence and resistance factors.

Results

Between 2015 and 2023 the median number of GAS infection per year was 106 (range:13-187 cases). In the same period, the median number of iGAS infection per year was 4 (range:1-10 cases). A decrease in the number of GAS infection was observed between 2020 and 2022, while in the first six months of 2023 110 cases were already reported.

The genomes of seven strains isolated from iGAS infections in 2023 (six from blood cultures and one from a cerebrospinal fluid) showed five different sequence types (ST), with three genomes belonging to ST28, a ST associated with increased mortality. Phylogenetic analysis of those three genomes showed that two out of three genomes clustered in the same branch.

All isolates had the following virulence genes: mac/ideS (a secreted cysteine protease), scpA (streptococcal cysteine protease A), sdaB (streptodornase B), smeZ (streptococcal mitogenic exotoxin Z), speB (cysteine protease,) and spyCEP (interleukin-8 protease). The only isolate which caused meningitidis had also superantigen speJ (streptococcal pyrogenic exotoxin J). Only one genome harboured ant, aph, erm, cat and tet resistance genes, involved in resistance to amikacin, clindamycin, erythromycin, and tetracycline.

Discussion and Conclusions

Our study showed a peak in the incidence in 2023, when compared to pandemic years. A similar peak was seen in 2016. Further investigation is required to determine the cause of the increased number of iGAS. Phylogenetic analyses showed that different ST were circulating in Pavia area. WGS analyses are crucial to characterize this pathogen at the genomic level, to evaluate the relatedness among the isolates and to detect the emergence of new lineages with the aim of studying GAS epidemiology and improving effective infection and control measurement.

217 - METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) CARRIAGE AMONG NASAL SURVEILLANCE SWABS AND MRSA AND MSSA RATE FROM CLINICAL SAMPLES OFTHE HOSPITALIZED PATIENTS OF "F. SPAZIANI" HOSPITAL, FROSINONE

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Methicillin resistant Staphylococcus aureus (MRSA) carriage among nasal surveillance swabs and MRSA and MSSA rate from clinical samples ofthe hospitalized patients of "F. Spaziani" Hospital, Frosinone

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Introduction:

Staphylococcus aureus (S.aureus) is one of the most widespread human pathogens worldwide. Antibiotic resistance to beta-lactams (methicillin-resistant S.aureus, MRSA) or glycopeptides (vancomycin-resistant S.aureus, VRSA) and its ability to survive in the biofilm state contributes to the difficulty of successfully treating S.aureus and requires the evaluation of adjunctive aspects of care. Our aim was to investigate MRSA positivity among the nasal surveillance swabs (nasal swabs) performed in the intensive care (ICU) and hematology units of "F. Spaziani" Hospital processed from June 1st 2023 to December 31st 2023 (7 months). In addition, we compared the rate of methicillin-sensitive S.aureus (MSSA) and MRSA among the S.aureus positive bacterial cultures of the hospitalized patients attending all the units of theHospital over the same period of time.

Material and Methods:

S. aureus detection was based on macroscopic examination through culture on Columbia Agar (BD). The bacterial colonies were identified by MALDI-TOF mass spectrophotometry (bioMérieux). Antimicrobial susceptibility was performed by Vitek2 (bioMérieux). All statistical analyses were performed with the SPSS v.20.0 for Windows.

Results and Discussion:

Four hundred and forty nasal swabs were performed in the ICU (n=219) and in the hematology unit (n=221). Fifteen nasal swabs were positive to MRSA (15/440, 3.4%: 5 in the ICU, 10 in the hematology unit) and 34 were positive to MSSA (34/440, 7.7%: 20 in the ICU, 14 in the hematology unit). Two VRSA werefoundfrom swabs ofthe ICU. No significant difference emerged by comparing MRSA or MSSA frequency between the two units (p=0.294, p=0.372). Nasal colonization by MRSA or MSSA does not seem associated to sex (p=0.992, p=0.113) or higher age (>60 yrs: p=0.237, p=0.715). Considering all the cultural requests (nasal swabs excluded) performed on the hospitalized patients, we found 98 patients (sex: 50 males, 48 females; median age: 72.5) who had a S.aureus infection: 42 patients with a MRSA infection (42/98, 42.8%) and 56 patients with a MSSA infection (56/98, 57.2%). Three isolates of VRSA were isolated from samples of different units.Among all the units of the hospital, no difference

emerged between MRSA and MSSA infection rate ($p>0.05$) except for the sore ward whose MSSA frequency (37/53, 69.8%) was significantly higher than MRSA (16/53, 30.2%) ($p=0.008$). No site of infection (e.g. blood, respiratory samples, wounds) showed a higher rate of infection by MRSA compared to MSSA ($p>0.05$).

Conclusions:

MRSA nasal colonization percentage rate seems quiet restrained, however a similar rate of MRSA and MSSA among S.aureus positive bacterial cultures in almost all the units of the Hospital suggests the need of extend and enhance MRSA surveillance.

219 - INVASIVE SALMONELLA ENTERICA INFECTION IN LOMBARDY REGION, 2006-2015

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Invasive Salmonella enterica infection in Lombardy Region, 2006-2015

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Introduction

Salmonellosis is a leading foodborne bacterial infection, responsible for 180 million (9%) of the diarrheal illnesses that occur globally each year. This infection involves all the population but especially young and elderly people. Typhoidal S. enterica (TS) serotypes are human-adapted and cause invasive extra-intestinal disease (typhoid fever), while nontyphoidal S. enterica (NTS) are characterized by a broader host range and usually cause self-limiting gastroenteritis. However, some NTS serotypes cause extraintestinal infections and bacteremia. Here, we present a retrospective analysis of salmonellosis cases occurred in Lombardy Region over a ten-year period.

Materials and Methods

We retrospectively analyzed data within salmonellosis surveillance activity in the Lombardy Region from 2006 to 2015. Information regarding the confirmed cases was obtained from the datasheets filled by hospitals or clinical laboratories. S. enterica isolates were serotyped using Kauffman and White's classification.

Results

Overall, 2952 S. enterica isolates were serotyped, and 135 NTS and 4 TS serotypes were identified. The most common NTS serotype was S. Typhimurium (27%, n=798), followed by S. 1,4,[5],12:i:- (21,4%, n=632), S. Napoli (17,3%, n=511), and S. Enteritidis (12,1%, n=356). These 4 serotypes accounted for 77,8% (2297/2952 cases) of the NTS infections during the considered period. Most of the cases were children aged 0-4 years old (37,6%, n=910). S. enterica was detected in the blood of 152 patients (invasive infection). S. Choleraesuis was the first serotype isolated in blood (38,2%; 58/152), with an invasive index (blood isolates/total of serotyped isolates) of 77,3%, followed by S. Napoli (18,4%, 28/152; invasive index 5,5%). TS serotypes were detected in the 15,8% (24/152; invasive index 50%) of blood samples. A pick of S. Choleraesuis cases occurred in the period 2010-2012 (70,6%, 53/75). S. Choleraesuis mostly affected adults aged > 64 years old (69,6%), 91,3% of those evinced a systemic infection. Otherwise, the other serotypes caused an invasive infection in 7,6% (27/356) of cases in the same aged population.

Discussion and Conclusions

Our data confirmed S. Typhimurium and S. 1,4,[5],12:i:- as the predominant serotypes in Lombardy Region, as well as in Europe. Moreover, this analysis raised attention on NTS serotypes S. Choleraesuis

and S. Napoli, involved in invasive salmonellosis. This could be explained by the fact that S. Choleraesuis and S. Paratyphi C share the same antigenic formula, and S. Napoli is phylogenetically related with S. Paratyphi A. We highlight the necessity of monitoring NTS serotypes to deepen their association with invasive infection and perform detailed molecular analysis to investigate their invasion mechanisms.

224 - INVESTIGATION ON THE POTENTIAL LINK BETWEEN SEXUALLY TRANSMITTED INFECTIONS AND PROSTATE CANCER DEVELOPMENT.

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Investigation on the potential link between Sexually Transmitted Infections and prostate cancer development.

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Introduction The aetiological role of sexually transmitted infections (STIs) in the development of prostate cancer has been widely studied. Chronic inflammation and atrophy caused by several microorganisms have been considered as possible mechanisms leading to prostate cancer. In particular, microorganisms that are able to establish a chronic intracellular infection can alter crucial cellular pathways such as cell cycle and apoptosis or promote DNA mutations causing genomic instability. These alterations lead to uncontrolled cell proliferation and cell transformation establishing a tumor microenvironment. We have investigated the prevalence of different sexually transmitted microorganisms (Chlamydia trachomatis, Neisseria gonorrhoeae, Ureaplasma parvum, Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma urealyticum, Gardnerella vaginalis, Candida spp, human alphaherpesviruses 1 and 2) in urine samples of patients affected by prostate cancer (PCa), benign prostate hyperplasia (BPH) and a borderline group of patients with atypical small acinar proliferation (ASAP) and prostate intraepithelial neoplasia (PIN), through multiplex real-time PCR. Materials and Methods A total of 167 urine samples of patients affected by PCa (n=96), BPH (n=60), ASAP and PIN (n=11) were collected (Urology Unit, AOU Sassari). The detection of the different microorganisms was performed simultaneously using three different multiplex real-time PCR. Results Of the 167 samples collected 40,4% resulted positive for at least one of the microorganisms. M. hominis, U. parvum and G. vaginalis represented the most common findings with 56,72%, 17,91% and 13,43% prevalence, respectively. Prevalence of multiple infections was 16,67%. The most frequent association detected was the co-infection of M. hominis and U. parvum (50%). Prevalence of M. hominis positives in borderline group was 36% while in BPH 28,3% and in PCa 17,70%. Discussion and Conclusions Recent evidences suggest STIs are associated with an increased risk of prostate cancer, the second leading cause of cancer death among men. In light of this, we investigated the potential role of a panel of STIs by assessing their prevalence in PCa, BPH and

borderline patients. M. hominis, U. parvum and G. vaginalis are the most represented (56,72%, 17,91%, 13,43%). Interestingly, the percentage of positives for M. hominis is higher in the group of patients with precancerous lesions (36%) compared to other groups. It is known that chronic intracellular infection established by M. hominis can have mitogenic and anti-apoptotic effects leading to the onset and progression of prostate cancer. Our results are consistent with previous findings that suggest a hypothetical role of M. hominis as a trigger in cancerogenesis.

226 - GENOMIC EPIDEMIOLOGY OF THE RAPID "POST-PANDEMIC" RE-EMERGENCE OF GROUP A STREPTOCOCCUS IN AN ITALIAN TERTIARY UNIVERSITY HOSPITAL

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Genomic epidemiology of the rapid "post-pandemic" re-emergence of Group A Streptococcus in an Italian tertiary University Hospital

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Introduction. Group A Streptococcus (GAS, Streptococcus pyogenes) is a human pathogen causing a wide spectrum of diseases, from pharyngitis to severe invasive infections. While the COVID-19 pandemic initially reduced GAS notifications, starting from 2022 an upsurge in GAS cases was observed globally.

Here we describe the heterogeneous nature of the rapid GAS spread in the Varese area.

Materials and methods. A total of 117 GAS isolates were identified by MALDI-TOF MS in a 7-month timeframe (May 2023 – January 2024). All isolates were characterized by their antimicrobial susceptibility profile and a subset of 34 isolates was further investigated by genomics, employing both short- (Illumina) and long-reads (Oxford Nanopore Technologies, ONT) sequencing. Various databases and bioinformatic analyses were used to reconstruct resistome, mobilome and virulome of the analyzed genomes, alongside with Genome-Wide Association Studies (GWAS) to highlight potential genotype-phenotype associations.

Results. The 34 isolates were collected from patients across multiple wards and hospitals in the Varese area. Most patients were male (18/34), with a median age of 40 years (range 0-91 years, STD

26.6 years). Approximately one-third of isolates (14, of which 11 bloodstream infections and one meningitis) were from patients admitted at the Emergency Room, 8 from the pediatric hospital (equally distributed between a familiar cluster in the neonatal intensive care unit and from pediatric wards), 5 from the otolaryngology ward (of which 3 abscesses), 5 from outpatients and 2 from surgery and gynecology wards.

Genomics identified nine different GAS sequence types (STs) and 11 different emm types. The most represented STs univocally correlated with a single emm type: ST28 (nine isolates) with emm type 1, ST36 (six isolates) with emm type 12, ST101 and ST52 (four isolates each), with emm types 89 and 28, respectively.

Four isolates carried resistance determinants for macrolides (erm 23S ribosomal RNA methyltransferases) and for tetracyclines (tet(M) ribosomal protection protein). No extrachromosomal element was identified in the Illumina/ONT hybrid assembly, but Tn916-family genetic elements were identified within composite integrative and conjugative elements. Conversely, preliminary GWAS analyses did not yield any relevant results.

Discussion and Conclusions. This genomic investigation of GAS in the Varese area highlights a mosaic of STs and emm types, some of which recur more frequently. No clear clue on invasiveness may be drawn from genomics, possibly suggesting the role of host-related factors. Although the diffusion of antibiotic resistant clones is still contained, an association of resistance determinants with mobile elements and distinct epidemiological clones was observed.

227 - GENOMIC CHARACTERIZATION OF ST410 OXA-181-PRODUCING ESCHERICHIA COLI ISOLATES FROM HEMATOLOGY PATIENTS IN S.ORSOLA UNIVERSITY HOSPITAL

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Genomic characterization of ST410 OXA-181-producing Escherichia coli isolates from hematology patients in S.Orsola University Hospital

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Background

The spread of carbapenemases-producing Enterobacterales (CPE) as multidrug-resistant healthcare-associated pathogens is an ongoing global concern. Italy is known to be endemic for KPC-producing Klebsiella pneumoniae since 2010, while in the last few years the epidemiology of CPE has become more complex, as the diffusion of OXA-48-like and MBL producing strains had been described in different hospitals. In the first trimester of 2024, we observed a rapid increase in the detection of OXA-48-like-positive Escherichia coli isolates in several hematological wards of S.Orsola University Hospital, in Bologna. The main aim of our study was to describe the epidemiological, microbiological and genomic features of these strains.

Materials and Methods

In this study, we included 4 clinical strains of carbapenem resistant OXA-48-like producing E. coli collected between 27 February to 17 March 2024. Three of these strains were isolated from blood cultures, one from urine culture. Genomic DNA was extracted by DNeasy Blood&Tissue Kit (Qiagen, Hombrechtikon, Switzerland). Libraries were generated using DNA Prep Library Preparation Kit (Illumina, San Diego, CA, USA). Sequencing was performed with Illumina iSeq100 platform. Paired-end reads quality was evaluated using FastQC software. Genomes were assembled using SPAdes v.3.15.4 and annotated with RASTtk. MLST evaluation was executed by comparing each genome against typing schemes deposited in the PubMLST database. Plasmids were assessed using PlasmidFinder. Stress response, antimicrobial resistance, and virulence genes were detected using AMRFinderPlus.

Results

Whole genome sequencing of E.coli isolates revealed that all belonged to ST410. Several antimicrobial resistance genes as carbapenemase blaOXA-181, ESBL bla CTX-M15 and colistin

resistance gene pmrB_Y358N, and virulence factors, including aerobactin synthase IucA, ferric aerobactin receptor IutA, NADPH-dependent L-lysine N(6)-monooxygenase IucD, NIS family aerobactin synthetase IucC, yersiniabactin ABC transporter ATP-binding/permease proteins YbtP and YbtQ were found in all isolates. Plasmid evaluation determined high level of homology, assessing the presence of Col156, ColKP3, IncFIA, IncFIB, IncX3 and IncX4 plasmids. Further studies on clonality and phylogeny are ongoing.

Discussion and conclusion

Our study showed that the sudden increase of OXA-48-like-producing E.coli clinical isolates in hematology wards in S.Orsola University was determined by the diffusion of a high-risk clone, belonging to ST410 and harbouring different antimicrobial resistance and virulence genes. The spread of this type of multi-drug resistant organism, able to cause clinically highly relevant infections in critical patients, needs to be addressed timely with infection control and antimicrobial stewardship measures, both driven by microbiological and genomic surveillance.

234 - ONE YEAR SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERALES: THE IMPACT OF WGS

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Title: One year surveillance of carbapenem-resistant Enterobacterales: the impact of WGS

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Introduction: Carbapenem-resistant Enterobacterales (CRE) are of serious concern worldwide since infections caused by CRE are difficult to treat. Whole genome sequencing (WGS) is useful to characterize bacterial isolates and provides a finer resolution than traditional genotyping methods for finding epidemiological links. Here we describe the genomic characteristics of CRE strains isolated within routine CRE surveillance at Fondazione IRCCS Policlinico San Matteo (HSM), an 900-bed Italian hospital, between October 2022 and September 2023.

Materials and methods: A total of 368 CRE strains isolated at HSM were sequenced on the Illumina MiSeq instrument. The first isolate per patient per resistance mechanism was chosen for the sequencing. Kleborate was used to determine Sequence Type (ST), and the presence of virulence and resistance genes for the genomes belonging to the Klebsiella genus. MLST, ResFinder and VirulenceFinder were used for other bacterial genera.

Results: 298 out of 368 isolates (81%) were carbapenem-resistant Klebsiella pneumoniae (CRKp). 34.9% of CRKp (N=104) were isolated in Intensive Care Units, 24.5% (N=73) in specialist medicine departments and 19.46% (N=58) in surgery wards. The other 45 CRKp were from general medicine, pediatric wards, and outpatients. 84.56% (N=252) were isolated from rectal swabs, while the remaining 46 were from infections. Most of the CRKp were KPC-producing (62.01%; N=185), of these, 154 strains harbored blaKPC-3 and 23 harbored blaKPC-2. NDM-producing CRKp were also reported, with blaNDM-1 (25.50%; N=76) as the major NDM type. blaVIM, blaOXA-48, and double-resistance blaKPC-blaNDM genes were also reported (3,02%, N=9). The most represented ST in strains harboring blaNDM-1 are ST11 and ST6668, with the latter which is a new clone of CC147 that had spread rapidly from April 2023 at HSM and in other hospitals in Pavia area. Nevertheless, ST307 remained the most frequently isolated clone throughout the year (Figure 1). No carbapenemase genes were identified in 10% of CRKp (N=30), despite their phenotypic resistance to carbapenems.

Discussion and conclusions: WGS of CRE should be included in routine surveillance because hospitalized patients commonly harbor CRE strains, with the possibility of developing difficult-to-treat infections and/or of transmitting CRE to other patients. Knowledge of the characteristics of circulating strains is a powerful weapon against antimicrobial resistance.

238 - RESPIRATORY VIRAL INFECTIONS IN HAEMATOLOGICAL PATIENTS: THE BENEFITS OF SIMULTANEOUS TESTING WITH ALINITY M RESP-4-PLEX

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Respiratory viral infections in haematological patients: the benefits of simultaneous testing with Alinity m Resp-4-Plex

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Introduction: Influenza virus infection is a common cause of self-limiting upper respiratory tract infection, but in haematological patients, who often suffer of more severe life-threatening infections, it progresses to the lower respiratory tract. However, not much data is available on the actual incidence, morbidity and mortality of viral respiratory infections in this patient cohort.

Material and Methods: In our virology laboratory 1704 haematological patients’ nasopharyngeal swabs were analysed from 10/11/2022 to 05/04/2024 using Polymerase Chain Reaction technology (PCR) according to the manufacturer’s instructions. Innovative multiplex molecular test (Alinity m Resp- 4 – Plex, Abbott) was performed to detect respiratory syncytial virus (RSV), influenza virus A/B (flu A/B) and SARS-CoV-2, to monitor viral infection, in febrile and asymptomatic patiens.

Results and Discussion: No positive swabs for RSV were identified, on the contrary, 180 positives for SARS-CoV-2, 31 positives for influenza A, 2 swabs for flu B were identified. The swabs belonged to 4 patients with flu A and 1 patient with flu B, 2 patients were positive for both flu A and SARS-CoV-2. Median age was 64 years, 2 females and 5 males. Patients with oncohematologic pathology were 5. Five patients had pneumonia, 4 with procalcitonin (PCT, Roche Diagnostics) suggestive of bacterial infection. Only two patients were treated with oseltamivir. No patient died, and upon resolution of the radiological picture of pneumonia, regardless of the result of the swabs, chemo or immunotherapy were provided.

Conclusions: Flu A/B in immunocompromised patients, in our experience, is mild and self-limiting, however, further studies need to be carried out with larger samples. Alinity m Resp-4-Plex's simultaneous testing aims to increase laboratory and hospital efficiency and decrease discomfort for the patient by collecting just one upper respiratory swab for the analysis of 4 viruses.

243 - PREVALENCE OF SYPHILIS INFECTION BEFORE AND AFTER COVID -19 PANDEMIC

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Prevalence of syphilis infection before and after COVID -19 pandemic

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Introduction

In the last ten years, the incidence of syphilis infection increased worldwide, although variations exist among countries. Being both sexually transmitted infections (STIs), syphilis and Human Immunodeficiency Virus (HIV) coinfection can be frequent, despite the incidence rates depend on the prevalence of each infection within the community along with individual risk factors.

Aim of this analysis was to evaluate the trend of syphilis infection in our hospital over the course of 5 years including COVID-19 pandemic.

Material and methods

A retrospective analysis of all cases of syphilis from January 2018 to December 2022 was carried out, including both patients receiving a first diagnosis as well as those with previous history of infection. Syphilis diagnosis was defined using both nontreponemal and treponemal tests. Nontreponemal test was rapid plasma reagin (RPR) test while treponemal assays included Treponema pallidum haemo-agglutination (TPHA) assay and enzyme linked immunosorbent assay (ELISA) for the detection of IgM and IgG antibodies. Active syphilis (AS) was defined with a TPHA in the positive ranges along with positive IgM and/or RPR titer, while positive TPHA with a negative RPR/IgM were considered follow-ups (FU). The study period was divided in pre- (2018-2019), during (2020) and post- (2021-2022) pandemic.

Results

From January 2018 to December 2022, a total of 15325 tests were performed including 1280 positive results. The 87% of the positive subject were male and the median (IQR) age was 43 (34-53) years, furthermore, 819 (64%) positive tests were of people living with HIV (PLWH). The number of positive results was 677/6925 (10%), 210/2429 (9%) and 393/5971 (7%) in the pre-, during and post-pandemic period, respectively. The ratio of AS/FU was 278/299 pre-pandemic, 114/96 during the pandemic and

249/144 post-pandemic. PLWH represented 63% of AS and 61% of FU pre-pandemic, 69% of AS and 54% of FU during, and 76% of AS and 55% of FU post-pandemic. Finally, within the AS, a small number of reinfections was observed, 6 in the pre-, 5 during and 22 in the post-pandemic, all in PLWH.

Discussion and conclusions

During the period considered a decrease in the positivity rate from pre- to post-pandemic and a reversal in the proportion of active infections versus follow-ups were observed.

The lower number of tests performed during 2020 could have delayed the diagnosis and/or the treatment of syphilis in the study population, possibly explaining the higher percentage of active infections, including reinfections, in the post-pandemic period. Finally, the persistence of a higher percentage of PLWH with a syphilis coinfection highlights the association that exists in the transmission of these two STIs.

246 - ACINETOBACTER BAUMANNII OUTBREAK TRACING USING THE FOURIER-TRANSFORM INFRARED (FT-IR) SPECTROSCOPY

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Acinetobacter baumannii outbreak tracing using the Fourier-Transform Infrared (FT-IR) spectroscopy

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Introduction: Acinetobacter baumannii is an opportunistic pathogen responsible for nosocomial infections in Intensive Care Units (ICUs). Nevertheless, there are overrepresented (clones) isolates of A. baumannii among those recovered from patients. This study aims to evaluate the reliability of Fourier Transform-Infrared Spectroscopy (FT-IR), a new typing method to assess clonality between A. baumannii strains compared with the genetic approach of Multiple locus variable number of tandem repeat analysis (MLVA).

Materials and Methods: 63 A. baumanni strains collected in 2022-2023 were analysed for this study. Antimicrobial susceptibility was assessed by broth microdilution. All the strains were tested for OXA-23 production (Coris) and PCR for armA and rmtB. MLVA was carried out for all strains considering ten VNTR markers to evaluate the clonality. The repeat unit size of VNTR above 9 bp was named large (L)-repeat VNTRs, whereas up to 9 bp were named small (S)-repeat VNTRs. The number of repeats in VNTR alleles for isolates of A. baumannii was estimated by subtracting the flanking region size from the amplicon size and then dividing by the repeat unit length. All the strains were also analysed by FT-IR. FT-IR analysis was performed with IR Biotyper® system (Bruker Daltonics, Germany) from strains grown on Columbia Blood Agar (37°C, 24h±1h). Data analysis of acquired spectra was performed considering only carbohydrates region (1200-900 cm-1) and applying hierarchical cluster analysis (HCA), Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA).

Results: A resistant profile was observed for all the antibiotics except colistin; all the strains were OXA-23 producers. 58 out of 63 harbour armA and none rmtB. Eight different patterns were obtained with MLVA. Four different big clusters were visualized with FT-IR, grouping some of MLVA. The two techniques were compared, and the agreement was high, corresponding to 92,6%. The discrepancy in the classification between MLVA and FT-IR dendrogram analysis was justified by evaluating the scatter plot. Strains with a slightly different MLVA pattern are included in the same FT-IR cluster and ranked at the edge of the FT-IR cluster.

Conclusion and discussion: MLVA had excellent discriminatory power, especially for short-repeat VNTR markers, for which rapid alterations could be significant when comparing epidemiologically related isolates over a long period. FT-IR can classify the cluster correctly and trace an ongoing

outbreak. To conclude, results obtained with FT-IR may offer a valid alternative to quickly identify a nosocomial outbreak than other molecular techniques, especially for user-friendliness and time savings, which allows performing real-time epidemiological surveillance.

252 - ANALYSIS OF MICROBIOLOGICAL INFECTIONS IN PEOPLE LIVING WITH HIV AND RELATIONSHIP WITH CD4 CELL COUNT

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Analysis of microbiological infections in people living with HIV and relationship with CD4 cell count

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Introduction

The impact of bacterial infections in people living with HIV (PLHIV) has been extensively reported. Bloodstream infections (BSI), respiratory infections (RI) and urinary tract infections (UTI) are more prevalent among PLHIV compared to the general population. Assessing immunological status is crucial in evaluating infections, as the severity of microbiological infections can be influenced by the patient's immune state. In PLHIV monitoring the CD4 count is key to identify progressive immunological decline that can lead to AIDS. Our study aims to assess CD4 count decline in PLHIV with BSI, RI, UTI and tuberculosis infection (TB).

Material and Methods

We conducted a retrospective evaluation of BSI, RI, UTI and TB in 2067 PLHIV who were either newly diagnosed, regularly followed up, or hospitalized at the Policlinico Umberto I in Rome, from January 2018 to January 2023. Bacteria were isolated on standard culture media (BD BBL™, Italy) and identification was performed using the Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Biotyper (Bruker Daltonics Inc., Germany).

In PLHIV with BSI, RI, UTI and TB, absolute CD4 count (cells/mcL) were performed by cytofluorometric analysis of whole blood samples (BD Biosciences, FACSLyrics) within 100 days before or after the acute microbiological event.

Results

Out of a total of 2067 PLHIV were documented: 63 (3%) BSI, 46 (2,2%) RI, and 67 (3,2%) UTI. Interestingly, among these, 67% with BSI, 43% with RI, and 52% with UTI were virologically suppressed at the time of infection. Additionally, 11 individuals tested positive for TB. Among them, 2 had undetectable HIV viremia.

Median value of CD4 cell count in PLHIV with UTI, RI, BSI and TB was: 428 cells/mcL, 226 cells/mcL, 149 cells/mcL, 109 cells/mcL, respectively. PLHIV with UTI showed higher CD4 count than BSI (P=0,0134) and PLHIV with RI had higher CD4 cell count than TB (P=0,0121). As expected, PLHIV with TB had the lowest median CD4 count.

Discussion and Conclusions

Despite the success of antiretroviral therapy, hospitalization and severe infections remain a concern among PLHIV. The CD4 count is a critical parameter in HIV management, serving as a reliable indicator of a patient's immune status and guiding clinical treatment decisions. In addition to examining the specific microbiological features of the infection, it is crucial to assess the immunological status. These data can provide insights into the burden of bacterial infections in this population, identify any evolving trends or patterns over time, and inform strategies for prevention, diagnosis, and management.

253 - MONITORING THE TRANSMISSION AND HEALTHCARE BURDEN OF RESPIRATORY VIRUSES IN EMERGENCY DEPARTMENTS

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Title: Monitoring the transmission and healthcare burden of respiratory viruses in emergency departments

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Introduction. Emergency department (ED) data can be used to monitor epidemic trends and identify new outbreaks of respiratory infections. However, their potential remains to be explored. Here, we provide a comprehensive analysis of the circulation of respiratory viruses identified in patients with influenza-like illness (ILI) admitted to an ED in the Lombardy region (Italy) in 2023-2024, estimating the transmissibility of these viruses and predicting their burden in EDs. Materials and methods. We analysed records of ED visits in the region (population ~10M) who underwent virological testing between September 2023 and March 2024. We estimated the daily net reproduction rate Rt for SARS-CoV-2, influenza virus, respiratory syncytial virus (RSV), metapneumovirus, parainfluenza virus, rhinovirus and adenovirus. ARIMA and dynamic regression models informed by real-time Rt estimates were used to predict the number of visits caused by each respiratory infection at 1 to 4 weeks. Results. 8,010 respiratory specimens from as many ILI cases (median age: 47; IQR: 4-75) were analysed; 48.5% tested positive for at least one of the viruses considered. In autumn, SARS-CoV-2 and rhinovirus accounted for about 80% of identified respiratory infections. In December, influenza virus type A was the main cause of a marked increase in ED visits, with an estimated peak Rt of 1.5-3.5. RSV contributed significantly to the number of ED visits during winter (especially in children <5 years), with a peak Rt of 1.4-2.3. Forecasts at 14 days resulted in a mean absolute error of <6 cases for each virus. Dynamic regression models incorporating Rt improved forecast performance during periods characterised by sudden fluctuations in case numbers. Discussion and conclusions. ED data can

provide timely information for monitoring the circulation of respiratory viruses and forecasting their impact on health systems, complementing the information on respiratory infection burden provided by community surveillance system.

256 - VARICELLA ZOSTER VIRUS ANTIBODY TITER IS NOT A GOOD MARKER OF THE CLINICAL PROTECTION DRIVEN BY CHICKENPOX VACCINE

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Title: Varicella zoster virus antibody titer is not a good marker of the clinical protection driven by chickenpox vaccine

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Background: The introduction of mandatory Varicella-Zoster Virus (VZV) vaccination during childhood, integrated as a compulsory measure in Italy in the National Vaccine Prevention Plan (PNPV) in 2017, has substantially reduced the overall incidence, complications and childhood hospitalizations associated with this infection. Our aim was to investigate the possible impact of vaccination on the overall immune response to VZV infection by comparing seroprevalence and antibody levels in pre- and post-vaccination era.

Materials: A retrospective analysis was carried out on 10,921 serum samples from patients that underwent screening and quantification of VZV-specific IgG at Bambino Gesù Children Hospital from January 2013 to December 2022, by using the chemiluminescence immunoassay (CLIA) technology.

Results: Overall, we observed a VZV seroprevalence of 79.7% in our general population. Median age was 19.3 years (IQR: 7.7-32.7). Individuals were classified into 9 age groups (Table 1). Seroprevalence showed an increasing trend starting in the younger age groups: <1 year, 49,4%; 1-5 years, 53,4%; 6-9 years, 68,3%; 10-14 years, 78,1%; 15-19 years, 86,9%; 20-39 years, 91,4%; 40-64 years, 94,1; 65-71 years, 98,4; >74 years, 96,0%.

Among the overall serum, 2,445 were collected in the pre-vaccination era (2013-2016) and 8,476 in the post-vaccination era (2017-2022). Comparing VZV antibodies prevalence in the different age groups, no significant difference was found between the two eras. However, a notable decrease in antibody titers was observed in post-vaccination era, particularly among age groups <10 years (Table 2). Moreover, we observed an 87% reduction in the median number of hospitalizations after vaccination era.

Conclusion: These results show a reduction in antibody titres in the post-vaccination era compared to the pre-vaccination era. These findings, taken in isolation, may initially raise doubts about the efficacy of the vaccine. However, a plausible explanation is that vaccine-induced immunity leads to the production of antibodies that are not easily detectable by standard VZV tests used in clinical practice. Furthermore, these tests do not distinguish antibodies produced by natural infection from those

induced by vaccination. Consequently, relying on conventional methods to measure VZV-specific Ig may not be the optimal choice for assessing the impact of vaccination.

Table 1. Study population

Total, N	10.921
Serum in the pre-vaccine era, N (%)	2445 (22,4)
Serum in the post-vaccine era, N (%)	8476 (77,6)
<1 year ,N (%)	318 (2,9)
1-5 years ,N (%)	1857 (17)
6-9 years ,N (%)	1111 (10,2)
10-14 years ,N (%)	1352 (12,4)
15-19 years ,N (%)	893 (8,2)
20-39 years ,N (%)	4068 (37,2)
40-64 years ,N (%)	1146 (10,5)
65-74 years ,N (%)	126 (1,2)
>74 years ,N (%)	50 (0,5)

Table 2: The median VZV-specific IgG titre levels (IU/mL) for each age group in the pre- and post-vaccination era. The last column shows the p-value data comparing both eras within each age group.

	2013-2016 Era			2017-2022 Era		
Age (year)	Median Title IgG (UI/mL)	IQR	Age (year)	Median Title IgG (UI/mL)	IQR	p
<1 y (N=39)	688.9	388.4-1218.0	<1 y (N=253)	356.9	173.8-755.6	0.003
1-5 y (N=174)	1086.5	397.6-1950.0	1-5 y (N=1,471)	595.1	275.4-1394.0	<0.001
6-9 y (N=131)	1305.0	556.7-2177.0	6-9 y (N=917)	949.2	458.8-1874.5	0.038
0-14 y (N=156)	1245.0	513.7-1963.5	10-14 y (N=1,144)	1063.5	563.1-1811.0	0.702
5-19 y (N=103)	1414.0	717.3-2068.0	15-19 y (N=772)	1106.0	600.9-1942.0	0.281
0-39 y (N=980)	1453.0	736.2-2032.0	20-39 y (N=2,977)	1136.0	669.5-1773.0	<0.001
0-64 y (N=310)	1524.0	780.1-2078.0	40-64 y (N=808)	1217.5	696.7-1915.0	0.008
65-74 y (N=32)	1352.0	813.9-2003.5	65-74 y (N=93)	1272.0	784.9-1868.0	0.732
>74 y (N=9)	1536.0	1075.0-2275.0	>74 y (N=8,476)	1459.0	772.7-2453.0	0.567

272 - SEROLOGICAL POSITIVITY OF TREPONEMA PALLIDUM IN TERTIAL ROME HOSPITAL: A RETROSPECTIVE EPIDEMIOLOGICAL STUDY IN LAST FIVE YEARS (2019-2023)

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Background: Sexually Transmitted Infections (STIs) are a large group of widespread infectious diseases worldwide, which can cause acute symptoms, chronic infections, and serious complications, and represent one of the most common global health problems. Syphilis is a systemic disease caused by the spirochaete Treponema pallidum (TP) and is one of the oldest known diseases for which curative and inexpensive treatment is available. In absence of animal reservoirs, syphilis should be an eradicable disease, but multiple concerted efforts to eliminate syphilis have failed, related to the ambiguous sexual behaviour of many people. Moreover, HIV infection can modulate the clinical presentation and the clinical and serologic response to syphilis treatment. Serological TP exams are performed routinely in many laboratories. Epidemiological monitoring is necessary for prevention and educational strategies especially among young people. Materials and methods: This observational study was performed at the Virology Unit of Tor Vergata Hospital, Rome, Italy (January 2019-December 2023) on blood samples collected for TP assays. Demographic data, diagnosis of TP by Venereal Disease Research Laboratory (VDRL) and Haemoagglutination Assay (TPHA, qualitative and quantitative), and serological positivity for HIV-1/2 (Combo test and Immunoblotting confirmation), and detection of other sexually transmitted pathogens were available. Exclusion criteria were age < 18 years old; data missing. Results were tabulated and descriptive and inferential analyses carried out. A p value < 0.05 was considered statistically significant. The study was conducted according to the Helsinki Declaration. Results: 20904 subjects were included in the study with 29352 reports diagnosing TP and/or HIV infection (January 2019-December 2023). Among them, 9979 were men, 10863 women and 62 with undefined sex. Mean age was 46.3 ± 15.8 years. Most of the enrolled subjects were outpatients (13428 subjects). Among the subjects who performed only the serological test for TP, 8% were positives. Correlation studies showed that nine patients were positive for both TP and HIV-1 (confirmed by immunoblotting) and two presented neurosyphilis. The same subjects also showed positivity for other sexually transmitted pathogens such as high-risk papillomavirus. Discussion and conclusions: STIs are still among the most common global health problems. Syphilis has revived interest both in developing and developed countries, especially in people living with HIV. In our epidemiological study TP infection is present in 8% of the screened population, especially in high-risk subjects that often are coinfected by other sexually transmitted agents. Serological screening may help identifying people at risk of infection.

273 - VIROLOGICAL SURVEILLANCE OF HUMAN WEST NILE VIRUS INFECTIONS IN LOMBARDY, JUNE-SEPTEMBER 2023

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VIROLOGICAL SURVEILLANCE OF HUMAN WEST NILE VIRUS INFECTIONS IN LOMBARDY, JUNE-SEPTEMBER 2023

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Introduction: West Nile Virus (WNV) is the main autochthonous arbovirus circulating in Italy. The Complex Structure of Microbiology and Virology, Fondazione IRCCS Policlinico San Matteo di Pavia, as Regional Reference Center for the diagnosis of arbovirosis is actively involved in the human surveillance of WNV. The aims of this study were to: i) carry out virological and epidemiological analysis of human cases of WNV; ii) understand the best biological materials and the most useful and informative tests to be used for the diagnosis of WNV infections.

Materials-Methods: In the period from 1 June to 30 September 2023, 394 suspected cases of WNV infection were investigated. Biological materials such as serum, plasma, liquor and urine have been investigated using molecular and serological methods. Direct analysis for the diagnosis of WNV involves the detection of viral RNA by Real time RT-PCR virus specification and a Hemi-nested RT-PCR Flavivirus. The indirect analysis was carried out by research of the antibodies IgM and IgG anti-WNV.

Results: 1June-30 September 2023, 394 cases of suspected WNV infection were investigated, of which 60/394 (15%) were positive while 334/394 (85%) were negative. The median age of positive patients was 72 years, ranging from 12 to 99 years. Of the 60 cases of WNV, 42 (42/60, 70%) were patients with neuro-invasive disease from WNV (WNND) and 18 (18/60,30%) were patients with febrile syndrome

(WNF). From a diagnostic point of view, both in cases of WNF and WNND, in the symptomatic phase of infection, WNV RNA was found in about 50% of urine samples, 20% of plasma samples and 15% of liquor samples. Compared to the serological diagnosis, about 90% of patients with neurological or febrile syndrome have been found WNV IgM in the absence or presence of IgG WNV.

Conclusions:For the diagnosis of WNV an integrated approach involving serological and molecular investigations was fundamental. The hemi-nested molecular test RT-PCR Flavivirus, identified the first autochthonous case of Dengue serotype 1 virus in Lombardy and was of crucial importance for differential diagnosis.

T09 NUOVI APPROCCI ANTIMICROBICI

15 - ANTI-STAPHYLOCOCCAL POTENTIAL OF CITRUS POLYPHENOLS: ANTIMICROBIAL AND ANTI-BIOFILM ACTIVITY, SYNERGY WITH BETA-LACTAMS AND LOW PROFICIENCY TO INDUCE RESISTANCE

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Anti-staphylococcal potential of citrus polyphenols: antimicrobial and anti-biofilm activity, synergy with beta-lactams and low proficiency to induce resistance

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Introduction. Staphylococcus aureus and Staphylococcus epidermidis are often antibiotic-resistant and can form microbial biofilms, leading to critical-to-treat infections. Polyphenols, plant-derived molecules with antibacterial and anti-biofilm activities, have been proposed as appealing candidates for treating drug-resistant and biofilm-related infections. Herein, we focused on the anti-staphylococcal activity of a polyphenol mixture extracted from citrus fruits (named B), its potential synergy with antibiotics, as well as proficiency to induce the development of bacterial resistance. Since liposomes were shown to be promising nano-vehicles for drugs in biofilm-associated infections, we additionally investigated the anti-biofilm potential of a formulation containing B encapsulated in liposomes (named OS).

Materials and Methods. Reference and clinical S. aureus and S. epidermidis strains were included in this study. Broth microdilution and time-kill experiments were used to test the anti-staphylococcal activity. Cytotoxicity was assessed by the hemolysis assay. The checkerboard assay was used to investigate the potential synergy with antibiotics. The ability to induce the development of resistance was verified propagating S. aureus for 10 transfers in the presence of sub-inhibitory B concentrations. Sub-inhibitory OS concentrations were used to evaluate the effect on biofilm formation by the crystal violet assay (CV). The eradicating activity of pure OS on mature biofilms was investigated by CV, plate count, and confocal laser scanning microscopy.

Results. B was active at low concentrations and displayed rapid bactericidal effects. No cytotoxicity on erythrocytes was observed. Interestingly, B showed synergistic activity with some beta-lactams against methicillin-resistant staphylococci. The exposure of S. aureus to sub-inhibitory B concentrations did not induce the development of resistance. We showed that OS reduced biofilm formation at sub-inhibitory concentrations. In addition, pure OS affected the biomass of mature biofilms, presumably acting on the biofilm matrix.

Discussion and Conclusions. Overall, our results support the use of B as promising option to manage staphylococcal infections. When encapsulated in liposomes, the extract additionally displays anti-biofilm activity, thus potentially representing a valid antibiotic-adjuvant in the treatment of biofilm-associated infections.

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33 - ALTERNATIVE STRATEGIES FROM AN EXOTIC SOURCE TO COMBAT ESKAPE PATHOGENS

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The ESKAPE pathogens, including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species, pose a global health threat due to their ability to resist antimicrobial drugs and evade the immune system. As a result, ESKAPE pathogens are responsible for more than 40% of infections in intensive care units and pose an economic burden, especially in low- and middle-income countries.

The scientific community has shown significant interest in using novel strategies to counteract the virulence of these pathogens. Anti-virulence drugs do not necessarily kill bacterial cells but prevent bacterial pathogenesis by targeting their virulence traits, and they can be used to combat the emergence of antibiotic-resistant pathogens. The use of anti-virulence strategies results in less evolutionary pressure, thus reducing the development of resistant strains. In this approach, the anti-ESKAPE drug administered would interfere with virulence factors instead of bacterial vitality, thus leading to the development of new strategies for the prevention and control of infections.

In this study, we focused on exploring the potential of Antarctic marine bacteria as a source of anti-biofilm molecules to combat ESKAPE pathogens.

In this work we analyzed the effect of supernatant derived from four different Antarctic marine bacteria, belonging to Pseudoalteromonas, Psychrobacter, and Pseudomonas genera, against a collection of 60 clinical ESKAPE pathogens. In particular, antibiofilm effects of Antarctic bacterial culture supernatants were examined on ESKAPE pathogens either during biofilm development by adding it to the medium at the beginning of the growth (pre-adhesion period), or after biofilm formation (mature biofilm).

Firstly, to exclude an effect of supernatants on bacterial viability, their antimicrobial activity was tested; obtained results did not highlight any antimicrobial activity on all ESKAPE pathogens. On the contrary, cell-free supernatants were able to prevent biofilm formation and promote the disaggregation of mature biofilm. Thus, obtained results have shown the great potential of Antarctic bacteria as producers of molecules capable of counteracting the phenomenon of biofilm formation of bacterial species of significant clinical interest. These findings contribute to the development of new strategies for preventing and controlling infections caused by ESKAPE pathogens.

These new compounds could represent the starting point for the identification of promising new drugs that can be used in synergy with conventional antibiotics for the eradication of ESKAPE-associated infections. Given the challenges, any potential solution must be explored.

34 - ANTIVIRULENCE POTENTIAL OF A CHIONODRACINE-DERIVED PEPTIDE AGAINST MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII CLINICAL STRAINS

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According to the World Health Organization, Acinetobacter baumannii is one of the most resistant pathogens belonging to ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa and Enterobacter spp.). A. baumannii is an opportunistic bacterial pathogen primarily associated with hospital-acquired infections (HAI). The main infections caused by this pathogen are pneumonia and ventilator-associated pneumonia (VAP), bloodstream infection; meningitis, osteomyelitis, skin, tissue and bone infections, and urinary tract infection.

The eradication of A. baumannii is very complicated for its ability to: i) adhere and form biofilm, ii) survive desiccation, iii) make motility on medical implantable devices. Among the different virulence factors, outer membrane protein (OmpA) biofilm-associated protein (Bap), type IV pili and Csu pili are mainly involved in biofilm formation. As already mentioned, another virulence factor is the bacterial motility; despite to A. baumannii has been originally classified as a non-mobile pathogen, it is able to perform two types of motilities: the twitching, led by type IV pili, and the appendages-independent surface motility. These factors are only some of the features that contribute to the multi-drug resistance of this pathogen. The increasing resistance to conventional antibiotics has become a global health crisis.

A novel promising strategy for developing new antimicrobial agents aims to inhibit virulence rather than bacterial viability. In the search of new antimicrobial therapies, antimicrobial peptides (AMPs) have been identified as new potential drugs to replace or integrate classical antibiotics, as they show a broad spectrum of activity against human bacterial pathogens.

In order to identify different protein scaffolds, we decided to focus our work on Antarctic icefish Chionodraco hamatus. Different mutants from the original peptide named chionodracine (Cnd) were successfully designed based on its scaffold to improve the antibacterial activity. In particular, the peptide named KHS-Cnd, showed high ability to kill ESKAPE pathogens. In our previous work, KHS-Cnd has been efficiently tested against some virulence factors of clinical P. aeruginosa isolated from cystic fibrosis patients.

In this study we tested the efficacy of KHS-Cnd against A. baumannii clinical isolates. In particular, we analyzed the minimal concentration of KHS-Cnd able to inhibit bacterial growth (MIC), biofilm formation (MBIC) and eradication (MBEC). Furthermore, the effect of KHS-Cnd on surface motility and twitching was also evaluated. In conclusion the synergistic effect of KHS-Cnd and conventional antibiotics was investigated.

35 - SCREENING OF A CHALCONE-BASED LIBRARY FOR ANTI-LEISHMANIA ACTIVITY

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Screening of a chalcone-based library for anti-Leishmania activity

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Introduction

Leishmaniasis is an endemic disease in nearly 100 countries and is characterized by high mortality rates. Current treatment for this parasitic disease presents several limitations such as toxicity, high expenses, method of administration, and the emergence of drug resistance. Thus, the development of new treatments for leishmaniasis is a priority in the field of neglected tropical diseases. The current study aims to investigate naturally derived products, particularly chalcones, as potential antileishmanial drugs.

Materials and Methods

The chemical synthesis of chalcones (n=12) involved a base-catalyzed Claisen-Schmidt aldol condensation reaction using acetophenone and aromatic aldehyde, followed by the subsequent addition of specific functional groups onto the main scaffold rings. Inhibition tests were carried out using reference strains of Leishmania donovani and L. infantum; extracellular promastigotes were incubated with the tested compounds for 72 hours and the IC50 (the concentration at which the compounds caused 50% inhibition of growth) was determined using the AlamarBlue assay. Additionally, the inhibitory effect of the compounds was assessed on the intracellular amastigote form of the parasite in infected human acute monocytic leukemia cells (THP-1). Infection rate was evaluated through microscopic observation of slides stained with Giemsa. Toxicity of the selected molecules on human cells was evaluated on THP1 cells, and the selectivity index (SI; the ratio between cytotoxicity and anti-Leishmania activity) for each compound was calculated.

Results

Four compounds were able to inhibit the in vitro growth of promastigotes at micromolar concentrations, with IC50 values ranging from 2.9 to 6.6 mM. Some of these molecules showed low toxicity on human cells, with values ≥ 300 mM. A preliminary investigation of the structure-activity relationship (SAR) of the newly synthetized chalcones was performed recognizing crucial chemical features involved in the antiparasitic activity. The calculation of the SI identified two molecules (AP3130 and AP3122) as promising leads. These two compounds exhibited good inhibitory activity

against the intracellular form of L. donovani, while only AP3130 showed good activity against L. infantum. Finally, a 3D molecular modeling analysis allowed to propose parasitic flavoproteins as possible targets of the investigated chalcones providing insights about the molecular basis of the antileishmanial activity exerted by these compounds.

Discussion and Conclusions

Two lead compounds showed good antiparasitic activity in vitro and can be considered as novel drug candidates against leishmaniasis. Further evaluation will focus on identifying the mechanism of action of these selected molecules.

43 - RESVERATROL, CHLORHEXIDINE AND BENZALKONIUM INHIBIT BIOFILM GROWTH IN ACINETOBACTER BAUMANNII

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Resveratrol, Chlorhexidine and Benzalkonium inhibit the biofilm growth in Acinetobacter baumannii

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Introduction

The persistence of Acinetobacter baumannii in the contaminated environment is supported by its tolerance to biocides and ability to growth as biofilm. The aim of the study was to analyze the susceptibility of A. baumannii biofilms to chlorhexidine (CHX) and benzalkonium (BZK) biocides and evaluate whether the natural monomeric stilbenoid resveratrol (RV) was able to modulate susceptibility to biocides of A. baumannii biofilms.

Materials and Methods

Anti-biofilm activity was determined by broth microdilution while biofilm formation and biofilm permormed were tested by crystal violet and tetrazolium salt reduction assay, respectively. RV, CHX and BZK combination activity against A. baumannii strains was assessed by a microboth checkeboard assay. Real-time RT-PCR assays were performed to study the gene expressions of Eps of A. baumannii ATCC19606 in the biofilm growth. All statistical analyses were carried out using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA, United States).

Results

CHX and BZK biocides at the concentration of ¼ MIC and ½ MIC alone or in combination dose-dependently inhibited biofilm growth of A. baumannii ATCC 19606, 4190 and 3909 strains assigned to distinct epidemic clonal lineages. The combinations of RV at 32 mg/L and ¼ MIC CHX and BZK showed a synergistic effect and completely inhibited biofilm formation in all A. baumannii strains. Similarly, the combination of 32 mg/L RV and ½ MIC CHX and BZK significantly inhibited air-liquid biofilm formation of A. baumannii ATCC 19606, 4190 and 3909 strains. The inactivation of AdeB and AdeJ RND Efflux pumps (Eps) in A. baumannii ATCC1960 increased the susceptibility to CHX and BZK alone or in the presence of 32 mg/L RV. Concordantly, CCCP increased the susceptibility to CHX, BZK and RV and dose-dependently inhibited biofilm formation in A. baumannii ATCC 19606, 4190 and 3909 strains. RV

at 32 mg/L inhibited basal and CHX-induced EP genes expression, while increased EP gene expression in the presence of BZK during A. baumannii ATCC1960 biofilm growth. In addition, CHX and BZK alone or in combination dose-dependently reduced preformed biofilm of A. baumannii ATCC 19606, 4190 and 3909 strains. The combination of RV with CHX and BZK additively decreased minimal biofilm eradicating concentrations in A. baumannii strains.

Conclusions

These results demonstrate that: i. CHX and BZK alone or in the presence of RV inhibit biofilm growth and preformed biofilm in A. baumannii; ii. Tolerance to CHX and BZK during biofilm growth is dependent on the activation of AdeB and AdeJ EPs; iii.The inhibitory effect of RV on biofilm formation is mediated by the inhibition of EP genes expression in A. baumannii.

45 - EVALUATION OF BROAD-SPECTRUM PIPERAZINE-BASED COMPOUNDS ABLE TO INHIBIT FLAVIVIRUS AND/OR SARS-COV-2 REPLICATION IN A LIVE VIRUS ASSAY.

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Evaluation of broad-spectrum piperazine-based compounds able to inhibit flavivirus and/or SARS-CoV-2 replication in a live virus assay.

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Introduction. Despite intensive work, no specific antiviral therapy is available for Zika or Dengue flaviviruses (ZIKV, DENV) and only 2 drugs (Nirmatrelvir, NRM and Remdesivir) are available against SARS-CoV-2. The aim of this work was to evaluate the in vitro activity of a set of newly synthesized compounds (CMPs) against ZIKV, DENV and SARS-CoV-2.

Materials and Methods. CMPs were designed with a piperazine ring as central core, using a privileged structure-based approach for the functionalization of both nitrogens. Two families of molecules were designed with 2-phenyl piperazine (1°family, 1-29) or unsubstituted piperazine (2°family, 30-51). CMPs were tested in a live virus cell-based assay to determine their antiviral activity. Once assessed the 50% cytotoxic drug concentration (CC50), lung A549 ACE-2 TMPRSS-2 (A549-AT) and hepatoma Huh7 human cell lines were treated with non-toxic doses of each CMP and challenged with SARS-CoV-2 (A549-AT) and ZIKV/DENV (Huh7) viral stocks at 0.001 MOI. Each experiment was performed in 2 independent runs including a mock control, a virus control and 2 reference CMPs (NRM for SARS-CoV-2 and sofosbuvir, SOF for flaviviruses). The inhibitory activity of each CMP was determined by measuring the expression of SARS-CoV-2 nucleocapsid and ZIKV/DENV envelope by immunodetection. Results were expressed as half-maximal inhibitory concentration (IC50) using a non-linear fit normalization curve. Selectivity index (SI) was defined as the ratio between CC50 and IC50.

Results. The median CC50 of the 1°family was 62 [22.7-200.0]µM in Huh7 and 138[36.5-200.0]µM in A549-AT. The 2° family showed a median CC50 of 400.0[112.9-400.0] µM in Huh7 and 130[89.4-400.0] in A549-AT. CMPs 24 and 26 were active only against ZIKV(IC50 2.5±1.4 and 9.6± 4 µM; SI 80.0 and 2.5 respectively). CMP 50 showed activity against ZIKV(IC50 2.7±0.7 µM; SI =148.1) and SARS-CoV-2(IC50 22.5±1.5µM, SI= 7.6); 9 CMPs were active against ZIKV(IC50 13.2[3.2-43.7]µM, SI 11.8[9.2-127]) and 4 of them (35, 39, 41 and 42) were active also against DENV (IC5029.3±14.3µM;SI=36.8). Globally,

compounds with an IC50<15μM progressed to further analysis to establish the interactions with the active site of the enzyme.

Discussion and Conclusions. CMP 50 displayed activity vs.SARS-CoV-2 and ZIKV. Despite the anti-ZIKV activity of CMP 50 was higher than SOF, its anti-SARS-CoV-2 activity was 500-fold lower than NRM. However, its low molecular complexity will allow further structure-based optimization. CMPs 41, 42 and 49 inhibited ZIKV with IC50<5mM similarly to SOF. Of them, 42 showed higher SI than SOF both for ZIKV and DENV. Since the lack of options for the treatment of ZIKV and DENV infections, these CMPs are promising for the development of a new class of pan Flavivirus agents.

47 - LICHEN SECONDARY METABOLITES AS POTENTIAL INHIBITORS OF VIRAL 3-CHYMOTRYPSIN-LIKE PROTEASE (3CLPRO)

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Lichen secondary metabolites as potential inhibitors of viral 3-chymotrypsin-like protease (3CLpro)

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Introduction. Despite advances in managing SARS-CoV-2 infections, the virus continues to spread rapidly, prompting interest in repurposing existing drugs and natural compounds as treatments. Promising are agents that target the SARS-CoV-2 3CLpro, an enzyme crucial for viral replication. Lichen secondary metabolites stand out due to their diverse biological properties, offering significant potential for therapeutic use against the virus. Materials and methods. The inhibitory effects of lichens against the 3CLpro are assessed through kinetic analyses by measuring the fluorescence intensity with a microtiter plate-reading fluorimeter using a fluorogenic substrate. Cytotoxicity effects are conducted on murine Sertoli TM4 cells. In silico analyses are performed to examine the interaction dynamics between lichens and the 3CLpro. 3) Results. Initial screenings have identified several lichen compounds as potential inhibitor of 3CLpro, with inhibition rates from 67% to 99% and Ki values between 0.67μM to 22.98μM. These compounds act as slow-binding inactivators, exhibiting both competitive and noncompetitive inhibition mechanisms, targeting the substrate-binding site and the enzyme dimerization interface, respectively. Molecular docking confirms the biochemical data. Protocetraric, salazinic and fumarprotocetraric acids fit well within the 3CLpro active site, forming stable covalent bonds with the catalytic cysteine145. Perlatolinic acid, identified as noncompetitive inhibitor, binds both the free enzyme and the enzyme-substrate complex, disrupting crucial interactions necessary for enzyme dimerization and activity. Cell culture and viability assays confirm that lichens do not exhibit effects on TM4 cells. Discussion and conclusions. Our findings not only enrich the existing body of knowledge on therapeutic strategies against SARS-CoV-2 underscoring the utility of lichen-derivatives in developing safe and effective treatments, but also add an important piece to the mosaic of their already numerous biological activities, highlighting their promise as a source of novel antiviral agents. A hallmark of this study is the employment of a dual inhibition strategy, an innovative approach to antiviral drug development. The identification of perlatolinic acid as a potent noncompetitive inhibitor targeting the protease dimer interface represents a pivotal advancement in developing innovative therapeutic strategies against SARS-CoV-2, offering a promising strategy to overcome viral mutation-induced drug resistance. Based on the outcomes of

kinetic, computational and cytotoxicity studies, it can be concluded that some examined lichen secondary metabolites serve as suitable scaffolds for developing effective inhibitors targeting the cysteine enzyme of SARS-CoV-2.

48 - N-OXIDES AS POTENTIAL ANTIMICROBIAL ADJUVANTS FOR THE TREATMENT OF INFECTIONS CAUSED BY MULTI-DRUG RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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N-oxides as potential antimicrobial adjuvants for the treatment of infections caused by Multi-Drug Resistant Staphylococcus aureus (MRSA)

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Introduction: In the last decade, antimicrobial resistance has emerged as a global emergency. Revitalising inactive antibiotics is currently the only way to go through this healthcare crisis and combining antimicrobial adjuvants with available antibiotics is turning out the most promising approach. Due to their low toxicity, eco-friendly features and their use in household and personal care products, N-oxides are good candidates, such as the commercial acyclic LDAO and TDAO and the cyclic N-oxides with 12, 14 and 16 carbon atoms, which are synthesized at the University of L'Aquila. Materials and methods: The in vitro antimicrobial effects of detergents were assessed by the microdilution method in 96-well microplates, following CLSI guidelines. The two-dimensional checkerboard microdilution assay investigated the in vitro interactions between antibiotics and detergents. Growth was quantified spectrophotometrically at 595 nm. Haemolytic activity on human erythrocytes was determined as the percentage of hemolysis induced by the detergents compared to control conditions. Cytotoxic effects of detergents on human peripheral mononuclear blood cells (PBMCs) were evaluated using the MTT assay. Results: The safety profile of the newly cyclic compounds is promising when compared to commercial surfactants. Although increased carbon chain length correlates with higher toxicity, all detergents showed cytotoxicity values well above their effective concentrations when combined with oxacillin. The compounds show an efficacious antimicrobial activity strongly related to the length of the carbon atom chain with MIC90 values ranging from about 5 μM to 625 μM. In drug–drug interaction assays, all surfactants act synergistically, restoring sensitivity to oxacillin in MRSA, with dodecyl acyclic and cyclic derivatives most effective. The most promising compound is C12NOX with an IC50 value in PBM cells well below the MIC value alone and in combination with oxacillin. Discussion and conclusions: This study investigated the adjuvant potentialities of L-prolinol N-oxide alkyl derivatives compared with the commercial N-oxides LDAO and TDAO, alone and in combination with therapeutically available antibiotics, against MRSA. N-oxide surfactants are good antimicrobial adjuvants, considered as "antibiotic potentiators" and "resistance breakers" by restoring sensitivity to oxacillin in MRSA. Due to their low or absent

cytotoxicity, their combination with antibiotics is a viable therapeutic option for treating topical infections sustained by MRSA, especially since resistance to amphoteric surfactants in pathogenic microorganism has not yet been reported.

53 - GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM OVAL SORRENTO LEMON (CITRUS LIMON): AN ECO-FRIENDLY APPROACH FOR COMBATTING VIRAL INFECTIONS

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Green Synthesis of Silver Nanoparticles from Oval Sorrento Lemon (Citrus limon): An Eco-Friendly Approach for Combatting Viral Infections

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Introduction. The burgeoning threat posed by viral infections underscores the imperative for innovative therapeutic modalities to safeguard human health. Nanomaterials have emerged as a promising avenue to meet this demand. Presently, the eco-friendly synthesis of silver nanoparticles (AgNPs) stands out as a method offering antimicrobial efficacy, safety, and cost-effectiveness. This investigation delves into the utilization of AgNPs) derived from the peel (LP-AgNPs) and juice (LJ-AgNPs) of the Oval Sorrento Lemon (Citrus limon), a staple crop in the Campania region. The antiviral potential of these nanoparticles was assessed against viruses belonging to the Picornaviridae, Coronaviridae, and Herpesviridae families. Materials and Methods. AgNPs were synthesized via a reduction method employing an aqueous solution of silver nitrate and an aqueous extract derived from Citrus limon peel and juice. The formation of biosynthesized AgNPs was ascertained utilizing a UV–Vis spectrophotometer. The morphology and concentration of AgNPs were characterized using Dynamic Light Scattering (DLS), nanoparticle tracking analysis (NTA), and Transmission Electron Microscopy (TEM). Cytotoxicity assessments were conducted across a range of concentrations spanning from 500 to 7.8 µg/ml on VERO-76 cells utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT). Furthermore, the antiviral activity was evaluated through pre-treatment, co-treatment, cell pre-treatment, and post-treatment tests against enveloped (HSV-1 and SARS-CoV-2) and naked viruses (PV-1), with a multiplicity of infection of 0.01. Plaque reduction assays and real-time PCR were employed to elucidate the antiviral potential of the tested compounds. Results. LP-AgNPs and LJ-AgNPs exhibited spherical morphology with respective diameters of 80 and 60 nm, alongside concentrations of 4.4×108 and 4.2×108 nanoparticles per milliliter (NPs/ml). MTT assay data indicated low cytotoxicity, with cellular vitality reaching up to 80% at the highest tested concentration. In the viral pre-treatment assay, complete inhibition of HSV-1 and SARS-CoV-2 was observed at concentrations of 250 µg/ml and 125 µg/ml for LP-AgNPs and LJ-AgNPs, respectively. This finding was corroborated by PCR data, which demonstrated a fold reduction of 0.6-0.8 in the UL54 and UL27 genes for HSV-1, and the Spike protein (S) for SARS-CoV-2, after NP exposure. Conversely, the lack of viral inhibition against PV-1 suggests a potential action of both NP types against the viral envelope.

Discussion and Conclusions. The findings of this investigation suggest that LP-AgNPs and LJ-AgNPs derived from Sorrento Lemon could present a promising, eco-friendly, locally sourced, and safe approach against viral infections.

56 - LSRK LIGANDS AS A WEAPON AGAINST ANTIMICROBIAL RESISTANCE

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LsrK ligands as a weapon against Antimicrobial Resistance

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Introduction

The increasing antibiotic resistance (AMR) crisis poses severe global public health and economic challenges, necessitating innovative antimicrobial strategies that surpass current drugs' limitations. Our study focuses on quorum sensing (QS), a bacterial communication mechanism crucial for controlling biofilm and virulence factor production. By disrupting QS, we aim to reduce bacterial pathogenicity and enhance antibiotic susceptibility. Specifically, we target LsrK, a key enzyme in AI-2 mediated QS, through both ligand-based and nature-aided drug discovery approaches.

Materials and Methods

We designed and synthesized novel AI-2 related compounds and screened them alongside potential LsrK ligands from our secondary metabolite library. These compounds were tested for their ability to inhibit biofilm formation in S. aureus and P. aeruginosa. We further characterized the best performers to confirm that their antibiofilm activity was mediated through QS inhibition rather than direct antimicrobial action. Advanced spectroscopic techniques like differential scanning fluorimetry, tryptophan fluorescence spectroscopy, circular dichroism, and Saturation Transfer Differences-NMR were employed to evaluate ligand-LsrK binding.

Results

Our results revealed several compounds that significantly inhibited biofilm formation with remarkably low MBIC50 values against both bacterial strains. In-depth biological evaluations confirmed that the antibiofilm activities were predominantly due to QS inhibition. This was further supported by the lack of significant bacterial growth inhibition and the selective quenching of AI-2 mediated QS in V. harveyi strains. The successful binding of our compounds to LsrK, confirmed by various spectroscopic methods, provided insight into the mechanistic aspects of QS inhibition.

Discussion and Conclusion

Our findings emphasize the potential of targeting QS through LsrK inhibition as a promising strategy against AMR. The significant reduction in biofilm formation underscores the efficacy of this strategy. Notably, these compounds demonstrate their antibiofilm activity primarily through QS inhibition rather

than by direct bactericidal effects further validates the QS-specific action of the tested molecules. The use of advanced spectroscopic techniques provided a robust framework for understanding the molecular interactions at play. Overall, these findings not only contribute to our understanding of QS modulation through small molecule inhibitors but also pave the ways for the development of novel therapeutic strategies against biofilm-associated infections. Such QS inhibitors hold the promise of becoming a part of the arsenal against bacteria, addressing the urgent need for innovative approaches to combat antibiotic resistance.

58 - REPURPOSING MK-8245, AN ANTI-DIABETIC MOLECULE UNDER CLINICAL INVESTIGATION, TO DISABLE QUORUM SENSING IN PSEUDOMONAS AERUGINOSA

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Repurposing MK-8245, an anti-diabetic molecule under clinical investigation, to disable Quorum Sensing in Pseudomonas aeruginosa

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Introduction: The escalation of antibiotic resistance alongside diminishing investments from pharmaceutical industries underscores the imperative to identify novel treatments against multidrug-resistant pathogens such as Pseudomonas aeruginosa (PA). Thus, repurposing existing drugs or drug candidates offers several advantages, such as known safety profiles, pharmacokinetics, and time-saving, in discovering new drugs to combat resistant pathogens. Quorum sensing (QS) inhibitors emerge as a hopeful opportunity, particularly in combatting PA, which relies on intricate QS pathways to orchestrate virulence factors. This study was designed to identify, among existing drugs or drug candidates, anti-virulence agents to treat PA infections.

Material and methods: Using an in silico molecular docking model, we identified potential molecules interacting with lactones C4-HSL receptor (RhlR) binding site within a library of 3000 FDA-approved or clinically tested drugs. The 17 most promising compounds were preliminarily screened for their ability to inhibit lactone-dependent signaling in PAO1, a PA laboratory strain, and thereafter the most active molecule was used to treat PAO1 cultures to evaluate: 1) bacterial cytotoxicity 2) lactones release by a bioluminescence assay 3) biofilm production using crystal violet 4) pyocyanins, elastase, and rhamnolipids secretion by colorimetric methods and 5) motility assessing swarming area. Moreover, the effect of the compound on lactone release, swarming motility, and pyocyanin production was assessed on 20 PA isolates from patients with acute respiratory tract infections.

Results: MK-8245 (40 μM) emerged as the most active compound, inhibiting by almost 60% the expression of QS regulated genes (rhlR, rhlI and rhlA)without biocidal effects on microbes. MK-8245 significantly reduced virulence factors production such as rhamnolipids (by 60%), pyocyanin (by 40%), elastase (by 25%) and swarming motility (by 25%) in PAO1. Moreover, MK-8245 significantly reduced PAO1 biofilm formation and showed additive effects with imipenem and aztreonam against biofilm

formation. Furthermore, MK-8245 significantly reduced lactone release (by 60%), piocyanin production (by 50%) and swarming motility (by 25%) in PA clinical isolates.

Conclusions: Our study underscores the potential of MK-8245 as an anti-virulence agent against PA acting via the inhibition of lactones QS signaling. The significant activity of MK-8245 on clinical isolates and its synergistic effects with imipenem and aztreonam solidify its potential as a new candidate for the treatment of acute PA infections, emphasizing the importance of our findings in the field of antibiotic resistance and drug repurposing.

60 - NOVEL THERAPEUTIC STRATEGIES AGAINST HUMAN PAPILLOMAVIRUS (HPV)-INDUCED CANCERS

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Novel therapeutic strategies against human papillomavirus (HPV)-induced cancers

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Introduction. High-Risk human papillomaviruses (HR-HPVs) are responsible for virtually all cases of cervical carcinoma worldwide and several other types of cancers, including anogenital and head-and-neck cancers. Despite substantial advancements in anti-HPV vaccination, there is still an urgent need for treatments for the multitude of already infected patients, since no specific anti-HPV drug is available yet. The E7 oncoprotein of HPV is one of the major drivers of carcinogenesis, being able to interact with and degrade several proteins involved in cellular homeostasis. Among them, E7 interacts with and leads to the proteasomal degradation of the cellular phosphatase PTPN14, which is an inhibitor of YAP, a well-established oncogenic determinant in solid tumors, and pRb, a crucial regulator of cell cycle progression and an oncosuppressor.

Materials and Methods. By using the co-crystal structure of HPV18 E7 with the C-terminal domain of PTPN14, we performed an in silico screening of small-molecule libraries to search for compounds directed against the C-terminal domain of E7. Hit compounds were then tested in an ELISA assay to assess their capability to inhibit the E7-PTPN14 interaction and by MTT cell viability assay in different HPV-positive and HPV-negative cancer cell lines, as well as non-tumoral cells as a control. PTPN14 and pRb rescue in HPV-positive cells treated with test compounds was also assessed by means of Western Blot and immunofluorescence experiments. Effects on YAP signaling were analyzed through Real-Time PCR. The antitumoral activity was investigated through clonogenic assays, transwell migration and invasion assays, wound healing assays, and 3D spheroid formation assays. Effects of compound treatment on apoptosis induction and cell cycle progression were assessed by FACS analysis.

Results. Starting from the hit compounds emerged from the in silico screening, we identified Cpd20 as a specific inhibitor of the E7-PTPN14 interaction. This compound was able to rescue PTPN14 protein levels in HPV-positive cells in a dose-dependent manner, affecting also YAP shuttling and downstream signaling. Moreover, Cpd20 was able to inhibit the proliferation, migration, invasion, and stem

potential of HPV-positive cells, showing also a promising broad-spectrum activity against different HR-HPV genotypes (HPV16, 18, 45, and 68). A second compound that emerged from the screening – Cpd28 – was shown to be involved in the disruption of the E7-pRb interaction, being able to restore pRb levels in treated HPV-positive cells. Treatment with this compound showed to induce apoptosis and affect cell cycle progression in HPV-positive cells, without affecting HPV-negative cancer cells. As well as Cpd20, Cpd28 was also capable of inhibiting the migration and proliferation of HPV-positive cells. Further characterization of this compound is still ongoing.

Discussion and Conclusions. In this work, we discovered and characterized two small-molecule compounds able to inhibit the protein-protein interactions (PPIs) between E7 and PTPN14 – Cpd20 – and between E7 and pRb – Cpd28. These compounds exhibit very promising antitumoral properties, paving the way for the development of new therapeutic approaches to treat HPV-associated cancers.

63 - ANTIMICROBIAL PROPERTIES OF GRAPE POMACE EXTRACT AGAINST FOODBORNE PATHOGENS

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Antimicrobial properties of grape pomace extract against foodborne pathogens

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Introduction: Foodborne pathogens which include Salmonella spp., Escherichia coli, Staphylococcus aureus and Bacillus cereus, are responsible of a food safety problem for the global community. In the last decade, research has focused on the discovery of new food additives and supplements through increased polyphenolic levels. Several studies have shown that polyphenolic molecules, derived from different botanical matrices, are able to exert significant in vitro antibacterial activity. The phenolic compounds responsible for the antibacterial activity in wine by-products are phenolic acids, flavonoids, tannins and coumarins. These molecules are enriched in grape pomace, which is a mixture of grape stems, peels and seeds and is the most important coproduct of winemaking. The present study aims to investigate the antimicrobial activity of grape pomace extract (GpE) against foodborne pathogens. Materials and Methods: The in vitro antimicrobial activity of the selected GpE was determined by the agar diffusion method. The susceptibility of foodborne pathogens to different GpE concentrations was determined by the broth dilution method, also in binary combination with the standard antibiotics, to obtain the values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). To evaluate the ability of the selected GpE to inhibit biofilm formation against selected bacteria, the Biofilm Formation Inhibition assay was performed. Results: The largest Mean Diameter of Inhibition Zone values were ranged from 16.67 to 21.17 ± 0.85 against S. aureus and from 8.67 ± 0.47 to 14 ± 0.82 against B. cereus, showing a remarkable antibacterial activity

against Gram-positive bacteria. The MIC and MBC values were in the range of 15 to 40 mg/mL against S. aureus, while the range was 20 to 50 mg/mL against B. cereus, and finally the values were in the range of 40 to 60 mg/mL against E. coli. The binary combinations with Vancomycin and Amoxicillin showed in vitro antibacterial activity against Gram-positive bacteria, vice versa the other binary combinations tested had no effect against Gram-negative bacteria. Interestingly, with increasing concentrations of GpE, an increment in the percentage of biofilm inhibition against Gram-positive bacteria was observed. Specifically, the maximum concentration tested was able to inhibit biofilm formation against S. aureus and B. cereus, with inhibition percentages of 79.93 ± 2.40% and 83.36 ± 1.99%, respectively. Conclusions: Overall, our results demonstrated the ability of GpE to exert strong antibacterial activity and it could be considered as a valid candidate for the development of natural antibacterial agents, and for the management and control of foodborne infections and resistant biofilm-related infections.

65 - THYME (THYMUS VULGARIS LAMIACEAE) ESSENTIAL OIL REPRESENTS A NEW PROMISING FRONTIER AGAINST FOODBORNE PATHOGENS

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Thyme (Thymus vulgaris Lamiaceae) essential oil represents a new promising frontier against foodborne pathogens

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Introduction: Foodborne illnesses represent a growing public health problem worldwide, affecting millions of people annually. These ailments arise from consuming tainted food, manifesting in diverse ways, from mild gastrointestinal discomfort to severe, life-threatening conditions. Various microorganisms like Salmonella spp., Listeria monocytogenes, Yersinia enterocolitica can induce foodborne diseases if products are not handled and preserved correctly. In the last decade, an extensive research has explored plant essential oils with broad-spectrum antimicrobial properties, aiming to enhance perishable food quality and shelf life. This study focuses on assessing the antimicrobial efficacy of Thyme (Thymus vulgaris L.) essential oil (Thy-EO), which showed potential in the treatment of bacterial infections and could represents a viable alternative as a natural food additive to improve the quality and shelf life of perishable items. Materials and methods: The in vitro antimicrobial effectiveness of Thy-EO against S. enterica, L. monocytogenes, and Y. enterocolitica was assessed using agar well diffusion assay. The sensitivity to escalating Thy-EO concentrations was

determined by measuring inhibition zone diameters. Moreover, the microdilution method was performed to assess Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. Bactericidal or bacteriostatic activity was also determined by time-kill assays up to 24 hours from exposure. Results: Thy-EO demonstrated noticeable inhibitory effects against all tested bacterial strains, evidenced by inhibition zone diameters at low EO concentrations. The antimicrobial efficacy against tested foodborne pathogens was also confirmed through quantitative assessments of MIC and MBC values. In particular, a consistent decrease in bacterial growth was observed with the tested concentrations, ranging from MIC to 4xMIC. Discussion and Conclusions: The results obtained from the in vitro microbiological assays suggest that Thy-EO, rich in bioactive compounds, are able to inhibit the growth of tested foodborne bacteria. These findings provide evidence to consider the tested essential oil as a promising source for the development of new, broad-spectrum, green food preservatives.

66 - A FIRST-IN-CLASS PYRAZOLE-OXAZOLE ENHANCED ANTIFUNGAL ACTIVITY OF VORICONAZOLE: SYNERGY STUDIES IN AN AZOLE-RESISTANT CANDIDA ALBICANS STRAIN, COMPUTATIONAL INVESTIGATION AND IN VIVO VALIDATION IN A G

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A first-in-class pyrazole-oxazole enhanced antifungal activity of voriconazole: synergy studies in an azole-resistant Candida albicans strain, computational investigation and in vivo validation in a Galleria mellonella fungal infection model.

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Introduction. C. albicans remains the most common species causing invasive candidiasis, thus requiring urgently a global action plan. Polyenes, azoles, echinocandins, nucleoside analogs, and allylamines are the first-line treatment for antifungal infections. The efficacy of azoles in the treatment of mycosis and systemic yeast infection is proven, but their prolonged use favours the occurrence of drug-resistance. Here we report the identification of a novel class of compounds arising from the screening of our in-house chemical library and behaving as novel promising antifungal agents. Material and methods. The chemical protocol employed for the synthesis of the newly conceived pyrazole-isoxazole derivatives is an ecofriendly methodology encompassing five high yielding steps and the use of green solvents and reagents where possible. The antifungal activity of compounds was determined on C. albicans ATCC 90028 and ATCC 10231 by broth microdilution method, while the checkerboard assay was used to assess the synergistic interaction between voriconazole (VRC) and compound 5b. Expression levels of the ERG11 gene was analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). The toxicity of compound 5b was evaluated using G. mellonella larvae. Molecular docking studies of both 5b and VRC, in combination or alone, into the C. albicans lanosterol 14α-demethylase (CaLDM) catalytic domain helped defining molecular determinants underlying our in vitro and in vivo results. Results and discussion. Among all the compounds synthesized, the derivative featuring an isoxazole moiety at position 4, namely compound 5b, showed a remarkable growth inhibition against the azole resistant C. albicans ATCC 10231 strain in combination with VRC and reduced the yeast-to-hypha morphological transition. At the transcriptional level, the qRT-PCR revealed the downregulation

of the ERG11 gene expression in C. albicans treated with the combination of 5b and VRC. An in vivo toxicity and efficacy assay using G. mellonella larvae revealed that the administration of compound 5b to larvae previously infected with C. albicans leads to increased survival, but the combination with VRC led to a much higher survival rate, indicating the two agents can work in synergy in vivo. Finally, the docking studies unveiled the unique ability of the synergistic combination of compound 5b and VRC to provide a complete occupancy of all the lanosterol 14α-demethylase sites, namely the substrate entry channel (SEC), the heme-containing active site, and a putative product exit channel (PPEC). In silico assessment also highlighted the druglike nature of our newly conceived derivative 5b, thus paving the way to a further optimization of this new class of synergistic agents for antifungal therapy.

69 - INNOVATIVE THERAPEUTIC STRATEGIES AGAINST HELICOBACTER PYLORI: IN VITRO EFFECT OF NEISSERIAL MEDIATORS

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Innovative therapeutic strategies against Helicobacter pylori: in vitro effect of neisserial mediators.

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Introduction: Helicobacter pylori (H. pylori) colonizes the gastroduodenal mucosa in approximately half of the world's population, with outcomes ranging from commensal to pathogenic, including severe implications like gastric lymphoma and cancer. Treating H. pylori infection remains challenging, necessitating ongoing surveillance of resistance and exploration of novel treatments. Recent studies suggest H. pylori serological status influences the gastric microbiome, correlating gastric microbiota with oral origin and gastric cancer. This study evaluates neisserial mediators as anti-H. pylori strategies as antibiotic alternatives. Material and Methods: Commensal Neisseria species (N. flavescens, N. lactamica, N. cinerea) were screened for inhibiting H. pylori ATCC 43504 and two resistant clinical strains through overlay assays. Neisseria spp. colonies were inoculated into Tryptone Soy Broth (TSB) and incubated at 37°C overnight (ON). After centrifugation, the bacterial pellet was resuspended in phosphate-buffered saline (PBS) 1X and mixed with Columbia Blood Agar (CBA) for bottom layer formation. An equal volume of CBA was added for the top layer, followed by incubation at 37°C ON with 5% CO2. H. pylori strains were resuspended in Brain Heart Infusion with Yeast Extract (BHI-YE) and inoculated onto overlay plates and CBA as a positive control. Plates were incubated at 37°C under microaerobic conditions for 48 hours. H. pylori growth on the surface was then

resuspended in BHI-YE broth. The OD600nm of each suspension was measured, and the ratio between the Neisseria-containing plates and control plates' OD600nm was determined. Results: The overlay assay showed strong antibacterial activity of N. lactamica and N. cinerea against both reference and resistant clinical strains of H. pylori, conversely N. flavescens promoted H. pylori growth. Discussion and Conclusions: Preliminary findings suggest that secreted biomediators from certain commensal Neisseria species inhibit the growth of both reference and resistant clinical strains of H. pylori. Future research will assess the inhibitory action of other commensal Neisseria species'through overlay and co-culture assays.

76 - IN SILICO DESIGNED ANTIBODY-DERIVED PEPTIDES: STRUCTURAL CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY

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In silico designed antibody-derived peptides: structural characterization and evaluation of antibacterial activity

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Introduction

Microbial infections pose a major threat to public health in relation to the emergence and spread of antimicrobial resistance. To overcome this problem, adjunctive and/or alternative therapeutic strategies against infectious diseases are hypothesized, also exploiting natural or synthetic antimicrobial peptides of various origin. Previous research showed that antibody-derived peptides may exert antimicrobial and antiviral activities, as well as immunomodulatory effects through the activation of host immune cells. This study aimed to characterize newly designed antibody-derived peptides and their effect against representative Gram-positive pathogens, including multidrug-resistant strains, possibly involved in biofilm-associated infections.

Materials and Methods

An in silico analysis was conducted to design modified molecules starting from the sequence of previously described antimicrobial antibody-derived peptides. The antimicrobial activity of the modified molecules was evaluated against planktonic microbial populations by conventional colony forming unit assays, while their cytotoxicity was determined against epithelial cell lines. Structure-function relationships of the peptides were studied by circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy, and their interaction with bacteria was analyzed by confocal laser scanning microscopy analysis.

Results

The newly designed peptides showed a differential antibacterial activity against reference strains and clinical resistant isolates of Staphylococcus aureus, without toxic effects on mammalian cells. The mode of action appeared to be linked to a membrane perturbation mechanism, as suggested by killing kinetics assays, CD analysis and confocal microscopy observations. CD and NMR measurements

revealed a predominant random coil arrangement for all the tested peptides in aqueous solution, but differential structural conformations in the presence of bacterial membrane and cell wall components. The presence of a cysteine residue allowing the formation of a disulfide bridge proved to be essential for antibacterial activity.

Discussion and Conclusions

Overall, this study preliminary characterized newly designed, antimicrobial antibody-derived peptides. Further studies are needed to evaluate the in vivo activity of these molecules in suitable experimental models of infection, in view of their potential use as alternative therapeutic approaches.

79 - EVALUATION OF ANTIMICROBIAL AND CELL STIMULATING ACTIVITY OF THE ANTIBODY-DERIVED PEPTIDE L18R ON BIOFILMS GROWN ON 3D PRINTED RESIN RESTORATIONS WITH DIFFERENT POLISHING PROPERTIES

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Evaluation of antimicrobial and cell stimulating activity of the antibody-derived peptide L18R on biofilms grown on 3D printed resin restorations with different polishing properties

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Introduction

Microbial biofilms can form on various biological surfaces or medical devices. In the oral cavity, surfaces prone to biofilm infections include teeth, implants, and resin restorations. Here, biofilms trigger prolonged and dysregulated immune responses, leading to tissue damage, persistent inflammation, and implant failure.

Temporary implant-retained restorations are essential for supporting masticatory function and esthetics until the final restoration is installed. Even short-term restorations may serve as reservoirs for microorganisms, potentially affecting peri-implant tissues and triggering inflammation, endangering further procedures. This study aimed to investigate biofilm formation on acrylamide resins obtained by three-dimensional (3D) printing technology used for temporary restorations and to evaluate the impact of post-processing on microbial and human gingival fibroblast adhesion. Additionally, we aimed to investigate the antibiofilm, immunostimulant, and growth-promoting activities of the antimicrobial peptide L18R, synthesized from the sequence of the immunoglobulin heavy J2 gene.

Materials and Methods

3D-printed samples of two commercial resins underwent different polishing treatments. Surface characteristics and biological behavior were analyzed by contact angle measurement, microscopical observation (atomic force, confocal, and scanning electron microscopy), cellular viability assay, and qRT-PCR. Microbiological evaluation assessing monomicrobial or mixed species biofilm of Streptococcus sanguinis and Candida albicans was carried out in the presence and absence of L18R.

The impact of L18R on gene expression related to proliferation and immune response was analyzed using qRT-PCR.

Results

Surface characterization revealed differences in the microtopography among the polishing treatments, as well as in their wettability. Both the resins showed good biocompatibility, while one induced a different morphology on human gingival fibroblasts. This result was also supported by the analysis of ITα-6 and IL-6 gene expression. A different amount of S. sanguinis and C. albicans total biomasses was recorded in biofilms developed on untreated commercial or polished resins. The peptide L18R showed antibiofilm activity, absence of toxicity on human cells, and a positive effect for growth.

Discussion and Conclusions

A different biological behavior of human and microbial cells in contact with the 3D-printed resins and their polished surfaces was shown, as well as in the presence of the antimicrobial peptide. These findings hold promise for the development of novel approaches to microbial control at the transmucosal interface supporting implant success.

80 - TARGETED PROTEIN DEGRADATION AS AN INNOVATIVE ANTIVIRAL STRATEGY AGAINST SARS-COV-2 MAIN PROTEASE MPRO.

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Targeted protein degradation as an innovative antiviral strategy against SARS-CoV-2 main protease Mpro

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Introduction: To date, Proteolysis Targeting Chimera (PROTAC) technology has been successfully applied to mediate proteasomal-induced degradation of several pharmaceutical targets mainly related to oncology, immune disorders, and neurodegenerative diseases, but its exploitation in the field of antiviral drug discovery is still in its infancy. Recently, we described two indomethacin (INM)-based PROTACs displaying broad-spectrum antiviral activity against coronaviruses.

Methods: A novel series of INM-based PROTACs that recruit either Von-Hippel Lindau or cereblon E3 ligases was designed, synthesized, and characterized for their antiviral activity. The panel of INM-based PROTACs was also enlarged by varying the linker moiety. We applied antiviral assays to test the broad-spectrum anti-coronavirus activiy of the INM-based PROTACs, biophysical assays to demonstrate the interaction of INM and INM-based PROTACs to SARS-CoV-2 main protease Mpro and degradation assays in both transfected and infected cells to demonstrate the ability of the INM-based PROTACs to induce the specific degradation of SARS-CoV-2 Mpro.

Results: PROTAC 6 showed antiviral activity against SARS-CoV-2 in different cell lines and broad-spectrum activity against human endemic coronaviruses. Target investigation in both uninfected and virus-infected cells with PROTACs 5 and 6 demonstrated that INM-PROTACs do not degrade human PGES-2 protein, as initially hypothesized. Study of the biophysical binding of INM and PROTACs 3, 5, and 6 to SARS-CoV-2 Mpro by microscale thermophoresis (MST) indicated binding in vitro to SARS-CoV-2 Mpro of all compounds and molecular modelling studies supported the formation of a ternary complex between E3 ligase, PROTAC 6, and Mpro. Degradation assays in both transfected and SARS-CoV-2 infected cells indicated that PROTAC 6 is able to induce the concentration-dependent degradation of SARS-CoV-2 Mpro. Importantly, thanks to the target degradation, PROTAC 6 exhibited a 9- to 105-fold enhancement in antiviral activity with respect to INM depending on the cell line.

Conclusions: The identification of the first class of SARS-CoV-2 Mpro degraders with activity also in infected cells represents a significant advance in the development of effective, broad-spectrum anti-coronavirus strategies.

85 - ANTIBACTERIAL AND ANTI-BIOFILM ABILITY OF TUNABLE CIPROFLOXACIN DELIVERY THROUGH PERSONALIZED ELECTROSPUN PATCHES FOR TYMPANIC MEMBRANE PERFORATIONS.

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Antibacterial and anti-biofilm ability of tunable ciprofloxacin delivery through personalized electrospun patches for tympanic membrane perforations.

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Introduction: Tympanic membrane (TM) is a thin, oval-shaped tissue of the peripheral auditory system, that separates the outer ear from the middle ear cavity, able of transforming the incoming sound waves into mechanical vibrations. One of the mainly pathological conditions of TM is Chronic Suppurative Otitis Media (CSOM), in which there is a development of middle-ear biofilms that serve as reservoirs for antibiotic-resistant bacteria, leading to TM perforation and othorroea. Pseudomonas aeruginosa and Staphylococcus aureus are often identified as causative agents in the CSOM-affected middle ear. Antibiotic ciprofloxacin (CIP) has been found to be particularly effective in inhibiting the

microorganism growth, while showing no ototoxicity toward the inner ear. With the growing prominence of tissue engineering, several biomaterial- and biofabrication-based strategies, such as electrospinning (ES), are being investigated for reconstructing the perforated TM with a superior biological and mechano-acoustical response. In particular, ES meshes have emerged as versatile carriers for numerous bioactive compounds, serving various drug delivery applications. Therefore, this work aims to evaluate the antibacterial and anti-biofilm activity of TM patches with tunable CIP releasing abilities created with distinct ES modalities. Methods: The antimicrobial activity test of the 4 different CIP-loaded poly(ethylene oxide terephthalate)/poly(butylene terephthalate) (PEOT/PBT) electrospun patches, respectively named Mode I, II, III and IV, against S. aureus and P. aeruginosa, was carried out by disk diffusion test, by measuring the inhibition halo diameter; the antibiofilm activity was assayed by applying the bacterial suspensions onto the patches overnight at 37°C and then both by crystal-violet staining and by SEM analysis. Results: for both bacterial species, mode II and IV, with higher cumulative CIP release are the only modalities with an evident inhibition halo. In contrast, mode I, which had no drug content, and mode III, with a very low cumulative concentration, exhibited no detectable antibacterial activity. The SEM analysis showed that the formation of the S. aureus biofilm was inhibited in all the samples, regardless of the presence of CIP. Conversely, for P. aeruginosa, sparse small clusters were observed in modes I, II, and IV, whereas a more extensive biofilm formation was evident solely in mode III. The crystal violet staining method confirmed these data. Discussion and Conclusions: This work presents an innovative therapeutic solution that synergistically combines tissue engineering with tunable drug releasing capabilities, with the aim of minimizing clinical intervention in the treatment of CSOM-induced TM perforations.

88 - USE OF BACTERIAL SPECIES OTHER THAN STAPHYLOCOCCUS AUREUS TO ASSESS THE FILTRATION EFFICIENCY OF FACE MASKS.

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Use of bacterial species other than Staphylococcus aureus to assess the filtration efficiency of face masks.

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Introduction. Face masks play a pivotal role in reducing the spread of airborne pathogens. To assess their efficacy, it is essential to measure their bacterial filtration efficiency (BFE) in accordance with ISO standards. This requires the use of aerosols containing the Biohazard Group 2 bacterial species Staphylococcus aureus. The objective of the present study was to investigate whether microbial species belonging to the Biohazard group 1 could be exploited as a valid alternative in BFE tests in accordance with the standard EN ISO 14683 and whether the standard protocol could be modified for a safer and faster laboratory turnaround.

Materials and Methods. Tests were performed using two different surrogate species. The first was Rothia koreensis with a strain isolated from the human microbiota. The second species was Bacillus clausii, which was used as spores from a commercially available product (Enterogermina®, Sanofi). The reference comparator species was represented by the S. aureus strain ATCC 6538P. BFE tests were run following the protocol described in the EN ISO 14683 standard regulation using the GB-XF1000 apparatus (GBPI, China). A commercially available Type I standard face mask was used to measure the BFE. All tests were done in triplicate.

Results. In positive control runs, both B. clausii spore- and R. koreensis cells-loaded aerosol particles were observed to distribute along the plates of the Andersen impactor in a manner similar to those loaded with S. aureus. The mean particle size was found to be 2.80, 2.75, and 2.7 for S. aureus, B. clausii, and R. koreensis, respectively. The mean number of total viable “particles” plate counts by the different species was 2,270 (95% CI: 1,856–2,685, alpha = 0.005). These values fully satisfy the requirements indicated by the ISO standards (acceptability range: 1,700–3,000 impacts). The surrogate bacterial species were comparable in determining the percentage of BFE of a highly efficient Type I face mask (mean $\pm$ SD = 97.6 $\pm$ 0.75; C.I.: 96.4-98.8; alpha = 0.05).

Conclusions. The results of the study demonstrate that other bacterial species not categorised as a biohazardous organism within Group 2 could potentially be employed in the context of the ISO 14683 standard for the assessment of bacterial filtration efficiency. The study utilised two such organisms, Rothia koreensis and Bacillus clausii, which were found to be suitable for the purpose. The latter was employed in the form of spores as a cost-effective, commercially available suspension with a defined, standardized titer, thereby eliminating a consistent portion of the work required for inoculum preparation and saving at least one day in turnaround time.

92 - EVALUATION OF PROBIOTIC PROPERTIES OF LACTOCOCCUS LACTIS STRAINS ISOLATED FROM NATURAL WHEY STARTER CULTURES

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Evaluation of probiotic properties of Lactococcus lactis strains isolated from natural whey starter cultures

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Introduction: Lactic acid bacteria (LAB) are one of the most important health-promoting groups in the human intestinal microbiota, able of producing antimicrobial compounds, balancing the composition of intestinal commensal microorganisms and out-competing invading pathogens for ecological niches and metabolic substrates, and are classified as probiotics due to their ability to prevent and treat diseases and modulate immune responses in the host. Fermented milk, cheese, yogurt, and kefir are primary sources of probiotic LAB and their consumption has been shown to be associated with beneficial health effects. Among these, several Lactococcus lactis strains with probiotic properties have been proposed as a possible vehicle to deliver therapeutic molecules in the gastrointestinal tract. Anti-inflammatory properties have been described for some natural L. lactis isolates, whereas the ability to modulate the intestinal microbiota has been reported for an L. lactis strain obtained from a fermented milk products. This study aims to evaluate the probiotic potential of L. lactis strains, which were previously isolated from the above-mentioned food matrices. Methods: The L. lactis subsp. lactis strains A3, A5, B1, D1, D3, I1, I4, and I7, were isolated from natural whey starter cultures and characterized by their different RAPD profiles, then were analyzed for their tolerance to gastric and pancreatic juices, auto- and co-aggregation ability, hydrophobicity, haemolytic activity and antibiotic susceptibility. In addition, the ability of different strain to produce antimicrobial substances and to inhibit the adhesion of Enteroinvasive Escherichia coli (EIEC) and Salmonella Typhimurium on intestinal epithelial cells was evaluated. Results: The results highlighted the potential probiotic

properties of some strains, which showed high values of hydrophobicity and auto-aggregation and low values of co-aggregation with the tested pathogenic strains. In addition, studies of safety parameters, such as antibiotic susceptibility and haemolytic activity, confirmed the safety status of all strains under study. The results obtained show that L. lactis strains A3-A5-I4-I7 exert a protective effect on cells previously infected with EIEC or S. Typhimurium. Discussion and Conclusions: Due to the strain-specific effects of probiotic characteristics, there is great interest in isolating new probiotic candidates from natural sources characterized by high microbial diversity, such as natural whey starters cultures. Although further studies are needed to confirm the safety and probiotic properties of these strains, these results suggest that the new isolated strains of L. lactis could be considered for use as probiotics.

96 - SELECTION OF MYCOBACTERIUM TUBERCULOSIS ANTIGENS FOR DEVELOPMENT OF ANTI-TUBERCULOSIS ANTIBODY-DRUG CONJUGATE (ADC) STRATEGIES.

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Selection of Mycobacterium tuberculosis antigens for development of anti-tuberculosis antibody-drug conjugate (ADC) strategies

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Introduction

Tuberculosis (TB), an airborne disease transmitted by Mycobacterium tuberculosis (Mtb), claims about 1.5 million lives each year. The expected TB rebound due to the COVID-19 pandemic and the increasing emergence of multidrug-resistant Mtb strains make the development of innovative TB control strategies urgently needed. The Antibody-Drug Conjugate (ADC) technology, developed for treatment of cancer diseases or Staphylococcus aureus/Pseudomonas aeruginosa infections, may be a valid innovative approach for TB. The ADC strategy is based on the use of monoclonal antibodies (mAbs) connected to a cytotoxic drug/antibiotic, allowing for the precise drug delivery to the target. The conjugation of selected anti-TB drugs with mAbs specific for surface-exposed Mtb antigens may pave the way for the development of effective ADC molecules allowing the eradication of intracellular Mtb. The identification of the most suitable anti-Mtb payload (bedaquiline, pretonamid or linezolid), and the selection of the most efficiently cell-wall exposed antigen specifically targeted by mAbs are key prerequisites for the development of successful anti-Mtb ADC compounds. However, the selection of the best antigen/antibody candidates is hampered by the lack of commercially available mAbs directed against cell-surface Mtb proteins. In this study, a panel of recombinant Mtb antigens (expressing the hemagglutinin HA epitope, recognized by commercial anti-HA-mAbs) were evaluated for their suitability as potential candidates targeted by ADC molecules.

Materials and methods

Mtb antigens were selected according to their surface localization, requirement for Mtb survival/replication in the host, and conservation among major Mtb clinical strains. HA-tagged versions of the selected antigens were obtained by cloning the corresponding gene fused to the HA-encoding sequence into the pMV306-hsp60 integrative vector. The resulting recombinant plasmids were used to transform the Mtb H37Rv laboratory strain.

Results

Selected proteins included members of the mycobacterial specific PE/PE familes (PPE25, PPE51 and LipY) and cell-wall associated LprI, LpqH. Mtb H37Rv strains expressing HA-N terminus and HA-C-terminus tagged versions of these antigens were obtained. The efficiency of the antigen export to the cell-wall will be tested by western blot analysis of different subcellular fractions, while cell-surface exposure of the HA-epitope will be evaluated by proteinase K sensitivity assays.

Discussion and Conclusions

The identification of the recombinant antigens with the most efficient cell wall-exposure will allow the selection of recombinant Mtb strains to be used as targets for anti HA-antibody based ADCs. This research is supported by MUR funding within the PRIN2022 (Project no. 2022JTPP53).

97 - IMPLEMENTING AN EFFECTIVE MICROBICIDAL COATING TO TACKLE INDIRECT MICROBIAL TRANSMISSION

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Implementing an effective microbicidal coating to tackle indirect microbial transmission

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Introduction

The indirect transmission of pathogens via contaminated surfaces and fomites is a significant vector for spreading infections, particularly in densely populated environments like schools, airports, hospitals, and care homes. These locations are vulnerable to harboring a high microbial burden, inclusive of pathogenic entities that pose grave risks to individuals with compromised immune systems. Current strategies pivot on environmental and object disinfection and hand hygiene as primary defenses against indirect pathogen transmission. However, these conventional approaches are constrained by variable cleaning frequencies, thoroughness of execution, and their challenging enforcement in public domains. The imperative to innovate and create technologies that can substantially mitigate or eliminate microbial presence on frequently touched surfaces is underscored by these limitations. Responding to this call, we have implemented a biocompatible, cost-effective, and non-toxic antibacterial coating (Patent PCT/IB2020/055621) that exhibits unequivocal efficacy, eliminating 100% of the tested microorganisms in different types of materials. This groundbreaking invention represents a paradigm shift in reducing microbial loads on surfaces prone to environmental exposure, serving as a protective shield for medical apparatus and high-contact items.

Materials and Methods

Structural, morphological and thermal characterization of the microbicidal coating layed on different model surfaces (poly(vinyl chloride) - PVC , poly(methyl methacrylate) - PMMA, and aluminum composite) was carried out by X-Ray Powder Diffraction, Thermogravimetry and Scanning Electron Microscopy. Microbicidal activity of the coating was tested against Gram negative (E. coli) and positive

bacteria (S. aureus and MRSA), and against 229E coronavirus. The coating cytotoxicity was also evaluated in A375 epithelial cells by MTT assay.

Results

The resultant coating showed a 100% bactericidal activity against the all tested bacteria in all tested materials. Furthermore, it was highly effective in reducing the infectious load of the alphacoronavirus 229E. The cytotoxicity test showed no toxicity after a 24 h exposure time.

Discussion and Conclusions

The coating has good adhesion on different material surfaces, without altering its characteristics keeping its microbicidal activity. The outstanding outcomes pave the path for employing the antimicrobial coating in critical areas, notably in healthcare settings.

106 - TOWARDS THE DISCOVERY OF NOVEL SORTASE A INHIBITORS AS POTENTIAL ANTIBIOFILM AGENTS: A MOLECULAR MODELING APPROACH

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Towards the discovery of novel Sortase A inhibitors as potential antibiofilm agents: a molecular modeling approach

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Introduction

Antimicrobial resistance (AMR) is a global obstacle to public health and the sustainability of healthcare systems. Superbug bacteria are responsible for about 25% of infections and almost 30% of AMR-related deaths. The biofilm formation represents one of the most virulent factors involved in the insurgence of chronic and persistent infections like cystic fibrosis. Specifically, biofilm is a complex and structured community that adheres to surfaces and is embedded in a self-produced matrix of extracellular polymeric substances. Bacterial adhesion is a critical factor in bacterial pathogenicity, being a useful approach to inhibit the biofilm development inherently resistant to typical antibiotics, and the most promising target involved in this field is Sortase A (SrtA). The project aims to develop novel molecules able to interfere with the adhesion to the host tissue, targeting SrtA enzyme.

Materials and Methods

The 2KID X-ray crystallographic structure of SrtA enzyme from Protein Data Bank was used. Molecular docking recognition studies were carried out using Glide v. 6.7 software. For the virtual screening studies, we used a library from ZINC20 database. The library was prepared using the LigPrep tool. Molecular dynamics (MD) during the ligand-enzyme interaction was performed.

Results

Computation studies were performed, including molecular dynamics (MD) and molecular docking. The X-ray crystallographic model (PDB code: 2KID), was utilized to focus on the apo form of the target. Following protein preparation, MD simulations were conducted for 150 ns selecting the three models for the virtual screening (VS). The natural product database ZINC20 was downloaded and prepared for

docking studies. The average theoretical binding affinity, in terms of docking score, over the three conformations was used to select 24 best compounds. Subsequently, these compounds were thermodynamically evaluated through MM/GBSA calculations. 7 potential SrtA ligands were finally selected and purchased for the subsequent biological investigation.

Discussion and Conclusions

In this study, computational simulations were employed to identify potential inhibitors targeting bacterial SrtA enzyme. MD simulations allow to determine the three most representative conformations selected as models for VS. From ZINC20 database, 24 natural products were initially identified as SrtA ligands. Finally, to prioritize compounds with structural diversity, 7 compounds were purchased and are under to biological investigation. This integrative computational approach offers insights into potential Srta A inhibitors that can be further validated through experimental means. Indeed, the enzymatic assays for SrtA modulation were optimized and the potential activity of the compounds is ongoing.

107 - SEMISYNTHETIC DERIVATIVES BASED ON USNIC ACID AS PROMISING ANTIFUNGAL AGENTS

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Semisynthetic derivatives based on usnic acid as promising antifungal agents

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Introduction

In the drug discovery trajectory, nature has always played a key role in the hit identification process either leading to the discovery of biologically active metabolites or inspiring the design of new compounds. The usnic acid (UA) is a well-known secondary metabolite produced by different lichen species which is increasingly emerging for its antimicrobial, antifungal, and antiviral properties. Despite its high therapeutic potentiality in the field of the infectious diseases, UA suffers from poor solubility and systemic uptake along with hepatotoxicity issues. In an attempt to improve the drug-like properties of UA, we conceived the development of semisynthetic UA-based derivatives as novel antifungal agents.

Materials and Methods

The novel compounds were prepared starting from the UA enantiomers. Specifically, the (S)-UA was extracted using an already optimized procedure, while the (R)-enantiomer was purchased from commercial suppliers. The key synthetic step consisted in the formation of the enamine intermediates by reacting the optical active UA with the appropriate amine under microwave heating. All the compounds were obtained in sufficient amount and purity for the biological investigation. Water solubility was evaluated for the new class of compounds via HPLC methods in gradient elution at a flow rate of 1.0 mL/min using an XBridgeTM Phenyl. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined for all compounds on Candida albicans, Candida tropicalis, and Trichophyton rubrum.

Results

A set of 20 compounds was prepared derivatizing the single UA enantiomers with different amino acids and aliphatic substituents through a microwave-assisted approach. Water solubility of each compound was experimentally determined by using a standard HPLC protocol. Cytocompatibility

assay was performed for all compounds which were further interrogated for their antifungal activity and fungicide efficacy.

Discussion and Conclusions

In this work, we focused on the generation of a small series of novel semisynthetic compounds based on both (R)- and (S)-UA as promising agents against topical fungal infections caused by Candida albicans, Candida tropicalis, and Trichophyton rubrum. Almost all compounds displayed a good water solubility, along with an acceptable cytocompatibility profile. According to the biological studies on the three strains, the (S)-configured derivatives resulted to be the most potent compounds, showing antifungal activity in the micromolar and nanomolar range.

116 - CALCITERMIN-LOADED SMART GELS ACTIVITY AGAINST CANDIDA ALBICANS: A PRELIMINARY IN VITRO STUDY

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Topic: Nuovi Approcci antimicrobici

Calcitermin-loaded smart gels activity against Candida albicans: a preliminary in vitro study

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Introduction: Candida albicans is the most common fungal pathogen infecting many human tissues, including vaginal mucosa. In the search for innovative molecules with antifungal action, antimicrobial peptides seem to be promising. The aim of this study was thus to assess the antifungal activity of Calcitermin (Cal), a peptide of 15 aminoacids found in human nasal fluid previously reported to exhibit antimicrobial properties, especially in acid conditions. Moreover, toward a possible use for the treatment of vaginal infections, the antifungal activity of Cal was also assessed after incorporation in gels based on poloxamer 407 and xanthan gum.

Materials and Methods: The anti-Candida activity of Cal was assessed at pH 4.5 (to mimic vaginal environment) in two different conditions: Phosphate Buffer Solution (PBS) and Gel (G18/0.4). Based on preliminary tests, Cal was used at 0.125 – 0.062 – 0.031 mg/mL, against 104 CFU of the target C. albicans (ATCC 10231). Samples were incubated at 37°C for 3 and 24 hours, then aliquots were collected, diluted and seeded on agar plates for enumeration of Colonies Forming Units (CFUs). Positive and negative controls were also included in the assays, corresponding to PBS or gel without Cal, respectively with or without the yeast strain.

Results: After 3 h, Cal was significantly inhibiting C. albicans growth only at the highest concentration used (- 18%, $p < 0.01$). Instead, after 24 h Cal reduced C. albicans CFUs of 30% ($p < 0.01$), 35% ($p < 0.01$), and 51% ($p < 0.01$) at 0.031, 0.062, and 0.125 mg/mL, respectively, compared to controls. Notably, when Cal was incorporated in gel, the inhibition was already evident after 3 h, with a CFU decrease corresponding to -29%, -32%, and -46% ($p < 0.01$) at 0.031, 0.062, and 0.125 mg/mL, respectively. After 24 h of incubation, the activity of Cal was further increased, showing reduction values corresponding to 92%, 96%, and 98% compared to controls (all values, $p < 0.0001$), induced by 0.031, 0.062, and 0.125 mg/mL of Cal, respectively.

Discussion and Conclusions: The results showed a significant antifungal activity of the Cal peptide against C. albicans, also indicating enhanced activity when incorporated in gel, compared to Cal diluted in aqueous buffer, suggesting that the gel viscosity and chemical formulation may significantly improve the Cal action in vivo.

118 - NATURAL PEPTIDES FROM BROWN LEAVES OF MEDITERRANEAN SEAGRASS POSIDONIA OCEANICA AS TEMPLATE FOR IN SILICO DESIGN OF SYNTHETIC ANTIMICROBIAL PEPTIDES

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Natural peptides from brown leaves of Mediterranean seagrass Posidonia oceanica as template for in silico design of synthetic antimicrobial peptides

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The emergence of new infectious diseases and multi-drug resistant bacteria represent a serious global threat that requires the development of an innovative class of therapeutic molecules. In this context, antimicrobial peptides (AMPs) could play a prominent role in tackling fungal and bacterial infections. We have identified and characterized two novel peptide sequences (Posidoniasin-1 and Posidoniasin-2) and used artificial intelligence models such as AMPfun, dPABBs, Cell-PPd, HemoPI, ToxinPred, PeptideCutter and HLP, to evaluate their biological and physicochemical properties. To improve the antimicrobial and antibiofilm properties, we then created several modified sequences by inserting single and double mutations and replacing some acidic amino acids with hydrophilic and basic ones. The computational screening of the derivative sequences revealed high ability to affect harmful fungal strains, Gram-positive and Gram-negative bacteria. In conclusion, these results show that natural peptides from the brown leaves of P. oceanica, in combination with artificial intelligence, can provide a useful template for the design and development of non-toxic antimicrobial synthetic peptides for the treatment of drug-resistant diseases.

122 - INVESTIGATING THE EFFICACY OF AIR GAS SOFT JET PLASMA IN ERADICATING ORAL BACTERIAL BIOFILMS FOR THERAPEUTIC APPLICATIONS

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Investigating the Efficacy of Air Gas Soft Jet Plasma in Eradicating Oral Bacterial Biofilms for Therapeutic Applications

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Introduction. In recent years, Cold Atmospheric Plasma (CAP) has emerged as a new therapeutical approach that can deliver Reactive Oxygen and Nitrogen Species (RONS) for biomedical applications. CAP is a highly reactive ionized gas generated at room temperature, usually from noble gases (i.e., helium or argon), which flows into ambient air or is directly created in air. Recent studies investigate the use of CAP for the treatment of periodontal disease, which still represents a big challenge for management and novel strategies are needed. Therefore, the aim of this study was to evaluate the effectiveness of a new technology based on air gas soft jet CAP in eradicating biofilms formed by oral pathogens.

Materials and Methods. Biofilms of both Streptococcus mutans UA 159 and a complex mixture culture of saliva microorganisms - isolated from a patient with periodontitis - were developed. The saliva biofilm was characterized via next generation sequencing to determine the main bacterial phyla. Both biofilms were treated with the air CAP at a distance of 6 mm for different time points, corresponding to 30s, 60s, 120s and 180s. The Colony Forming Units (CFU) count, the XTT metabolic assay and the crystal violet assay were carried out to determine the effectiveness of air CAP in removing multi-species biofilms. To evaluate CAP cytotoxicity in eukaryotic cells, human Gingival Fibroblasts cells (hGF) were treated with CAP at the same distance and time points.

Results. A statistically significant reduction of both CFU count and XTT was detected after 60s of CAP application, while its application for 120s led to the eradication of the biofilms related to a reduction of -3/4Log10CFU/mL for saliva microorganisms and S. mutans, respectively. The live/dead assay and the CLSM analysis supported CAP effectiveness in reducing the thickness of the biofilm matrix and in killing the microorganisms inside the biofilms. Eukaryotic viability tests showed a low rate of cytotoxicity towards hGF cells.

Discussion and Conclusions. The present study demonstrated the ability of CAP treatment to eradicate biofilms developed by both S. mutans and saliva microorganisms, thus representing a potential innovative approach to fight oral microorganisms responsible of periodontal disease.

Further studies should be conducted on biofilms developed by additional saliva donors to support the potential of this strategy to counteract oral pathogens responsible for periodontal diseases.

A possible application of CAP in clinical field as a new tool to counteract antimicrobial resistance could be speculated, given that CAP has different mechanisms of action than currently used antimicrobial drugs.

125 - ASSESSING THE POTENTIAL OF N-BUTYL-L-DEOXYNOJIRIMYCIN (L-NBDNJ) IN MODELS OF CYSTIC FIBROSIS AS A PROMISING ANTIBACTERIAL AGENT

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Assessing the potential of N-Butyl-L-deoxynojirimycin (L-NBDNJ) in models of Cystic Fibrosis as a promising antibacterial agent

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Introduction: Iminosugars are sugar analogues in which an amino function replaces the endocyclic oxygen atom of carbohydrate backbone and have been investigated as agents able to control the onset of inflammatory events and bacterial infections characterizing cystic fibrosis (CF) lung disease. Pseudomonas aeruginosa is the major pathogen in the CF lung, and promotes an accelerated decline of pulmonary function. Our previous investigations highlighted the potential anti-inflammatory properties of l-NBDNJ in murine models of P. aeruginosa acute infection.

Materials and Methods: L-NBDNJ activity was evaluated in mouse models of chronic P. aeruginosa infection. Inflammatory cells recruitment and bacterial load were assessed in lung homogenates and in bronchoalveolar lavage fluids of C57Bl/6NCr mice six days post-infection. Cytokine/chemokine levels were analyzed using the Bio-Plex Protein Array System. The effect of L-NBDNJ was evaluated on gene expression of the P. aeruginosa RP73 strain by RT-qPCR analysis and on the adhesion of the RP73 strain to the A549 cells by cell adhesion/invasion assays.

Results: A slight reduction of inflammatory cells was observed in the murine model of P. aeruginosa chronic infection in presence of L-NBDNJ also accompanied by a significant decrease of bacterial load in the airways. Although its antibacterial activity was clearly detected in vivo, l-NBDNJ was not able to alter the in vitro viability of bacterial cells. Mechanistic insights into the observed activity revealed that L-NBDNJ down-regulates many bacterial virulence factors involved in the host response. After treatment with L-NBDNJ we observed a significant reduction of gene expression of aprA, encoding for alkaline protease, that inactivates complement system components and cytokines. We have found

that RP73 cells treated with l-NBDNJ expressed 5-fold lower levels of hcnA gene, encoding hydrogen cyanide synthase, and this reduction could improve lung function by limiting tissue destruction. The expression of exoS, exoT, exoY were significantly downregulated upon l-NBDNJ exposure. These exotoxins can cause cell death and also affect host defense, by blocking phagocytosis and bacterial clearance. Interestingly, human cell adhesion assays showed that the adhesion efficiency of RP73 cells was significantly affected (5-fold) by treatment with l-NBDNJ.

Discussion and Conclusions: These findings along with the observation of the absence of a bacteriostatic/bactericidal action in vitro of l-NBDNJ suggest the potential use of this glycomimetic as an anti-virulence agent in the management of CF lung disease.

130 - CHARACTERIZATION OF NOVEL ANTI-INFLUENZA COMPOUNDS WITH NANOMOLAR POTENCY AND SHOWING SYNERGIC EFFECTS WITH APPROVED DRUGS

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Characterization of novel anti-influenza compounds with nanomolar potency and showing synergic effects with approved drugs

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Introduction: Influenza viruses have always drawn attention as a thread for the public health. Influenza viruses are characterized by continuous viral antigenic drift, leading to ineffectiveness of the currently available therapeutic and prophylactic approaches and new viral strains resistant to the approved anti-influenza drugs are continuously emerging. Thus, there is an urgent need for novel and potent anti-influenza drugs. Our research group has two main goals in identifying new anti-Flu therapeutic strategies: i) addressing a novel, highly conserved viral target, that is the interaction between the viral RNA polymerase subunits PA and PB1, and ii) combining compounds targeting different viral cycle steps so that they can act synergistically against viral replication, decreasing the probability of drug resistance emergence. The PA-PB1 interaction is highly conserved among influenza A and B viruses and presents considerable drug-resistance barrier. A previous in silico screening led to the identification of a chemical structure capable to accommodate very efficiently in the PA cavity, i.e, the cycloheptathiophene scaffold, which thus represents a starting point to develop derivates with a higher binding affinity to the PA pocket. In addition, combination studies were carried out with the most promising compounds, in order to evaluate if they could have a synergic effect with currently available anti-Flu drugs with a different target, such as Oseltamivir.

Materials and methods: MTT assays were first performed to exclude possible cytotoxicity of the compounds both alone and in combination with available drugs. Anti-Flu activity was then evaluated by plaque reduction assays (PRAs). In addition, the ability of anti-PA/PB1 compounds to inhibit the protein-protein interaction and the viral polymerase activity was assessed by an ELISA-based interaction assay and by a cell-based minireplicon reporter assay, respectively. Moreover, the anti-PA/PB1 mechanism of action of the compounds was further validated through a cellular PA/PB1 nuclear translocation assay. To evaluate the presence of the synergic effect between anti-PA/PB1

compounds and some approved drugs, PRAs and virus yield reduction assays were performed. A Western blot was also carried out on infected cell extracts to analyse the expression of different viral proteins.

Results: Some cycloheptathiophene derivates showed potent anti-Flu activity in PRA and inhibitory activity in the ELISA-based PA/PB1 interaction assay. Some of these compounds were further tested in minireplicon assay and exhibited the ability to inhibit the activity of the viral RNA polymerase. In addition, their mechanism of action was validated by a PA/PB1 nuclear translocation assay. One of these compounds was tested also in combination with Oseltamivir in virus yield reduction assay and proved to have a synergic anti-Flu effect. In Western blot analysis on infected cell extracts, the expression of viral proteins was significantly decrease following the combined treatment with the cycloheptathiophene derivate and Oseltamivir.

Discussion and conclusions: Altogether, these results provide important information which will help us to develop more potent PA/PB1 interaction inhibitors. The most potent compounds will be taken forward into preclinical studies, by testing them in the chicken embryonated egg model both alone and in combination with approved anti-influenza drugs, in order to propose these novel compounds as possible enhancers of already existing therapeutic options.

141 - VIRUCIDAL ACTIVITY OF EPOXY RESINS

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virucidal activity of epoxy resins

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Background and aim. Sanitary surfaces play an important role in the transmission of nosocomial pathogens. Such evidence justifies the growing interest in identifying new materials to prevent and mitigate infectious risk. The objective of our project is to develop innovative nanostructured materials with antimicrobial properties to be used as collective protection measures in health settings. Here, we present the results of a study on the virucidal activity of 5 samples of 3 different commercial epoxy resins.

Materials and Methods. Three commercial epoxy resins have been used as polymer precursors: (1) Diglycidyl ether of bisphenol A epoxide (DGEBA, Elan Tech EC157), (ii) EPIKOTETM Resin MGS®; and (3) MC152. The epoxy resins have been prepared according to the resin/curing agent amounts indicated by the suppliers; resins 1 and 2 were also prepared with 10% w/w excess of curing agent (1b) and (2b). The virucide activity was tested against Herpes Simplex Virus type-1 (HSV-1), AdenoVirus type-5 (AdV5) and Human CoronaVirus OC43 (HCoV-OC43).

Protocol 1 had the aim to verify a virucide activity of resins by contact, while protocol 2 was set to assay whether the resins release virucidal molecule(s) in the medium. Protocol 1: the resin coupons were soaked in 2 mL of a viral suspension of known concentration, vortexed for 30 seconds and then

incubated for 1h and 24h. Protocol 2: the coupons were soaked in 1 mL of maintenance medium for 24h with continuous shaking. After 24 hours, the coupon was discarded and the supernatant incubated with the viral suspensions for 1h and 24h. In both cases, at the end of the incubation time the residual viral load was quantified by end-point titration.

Results. The sample 2b showed a remarkable inactivating activity against HSV-1 and, to a less extent, against HCoV-OC43, by both contact and release, although the inhibition is greater by release. In the case of the studies by contact the viral titre reduction was 0.7 Log after 1h and 3.5 Log after 24h and 1.7 Log and 3.4 Log in the release studies for HSV-1; for HCoV-OC43, a significant reduction was obtained only with 24h incubation, both by contact (1 Log reduction) and release (2.7 Log). No anti AdV activity was observed. The sample 2a showed a higher anti HSV-1 activity, but no activity against HCoV and AdV. The other resins did not display any significant antiviral activity.

Discussion and Conclusions. These commercial resins with intrinsic antimicrobial properties can constitute an ideal support in which to incorporate antimicrobial nanomaterials for the creation of high tactility objects (handles, furnishing elements, etc.) to be applied in the healthcare sector for the prevention of HAIs. Currently, we are testing the resins 2 with nanostructured clays included.

145 - ANTIBACTERIAL METABOLITES PRODUCED BY LIMONIUM LOPADUSANUM, AN ENDEMIC PLANT OF LAMPEDUSA ISLAND.

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Antibacterial Metabolites Produced by Limonium lopadusanum, an Endemic Plant of Lampedusa Island

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INTRODUCTION-The requests for new, effective, and eco-friendly tools to deal with the emerging human diseases have become urgent. Natural products are still an unmatched source of compounds with different biological activities. Plants are a rich reservoir of secondary metabolites with potential applications in food chemistry, agriculture, and medicine. In this context, the investigation of poorly studied endemic plants holds a great potential to unveil unprecedented chemistry and biology. The island of Lampedusa in Sicily has proven to be a rich source of plants and shrubs used in folk medicine. The Limonium genus is well known to produce bioactive secondary metabolites with antioxidant, antifungal and antimalarial properties, but until now there is no literature reporting the antibacterial activity of its secondary metabolites. Therefore, the extraction, purification and chemical characterization of the main metabolites produced from samples of Limonium lopadusanus Brullo was carried out. The antimicrobial activity of organic extracts of the plant and its metabolites was tested against important clinical pathogens.

MATERIAL AND METHODS- Organic extracts obtained from fresh aerial parts of L. lopadusanum Brullo were tested against methicillin-resistant Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, and the voriconazole-resistant Candida albicans starting from 1mg/mL. The antimicrobial tests were performed through standard broth-microdilution assay. CH2Cl2 extract was purified and five phenolic metabolites were obtained in the pure form; their structures were elucidated by a detailed MS and NMR investigation and by

comparison with the literature. The antibacterial effect of these secondary metabolites was assayed against Enterococcus faecalis.

RESULTS- The in vitro efficacy showed by the CH2Cl2 extract of L. lopadusanum in inhibiting the growth of E. faecalis appeared to be very interesting. All the metabolites isolated from the CH2Cl2 organic extract, except compound 5 which was obtained in too low amounts, were tested against E. faecalis and only compound 4 (erythrinassinate C) showed antibacterial activity.

DISCUSSION AND CONCLUSIONS- In summary, our pytochemical investigation of the bioactive antibacterial extract of L. lopadusanum afforded five metabolites, all belonging to the class of phenolic derivatives. We isolated two extremely rare metabolites, erythrinasinnate C and ligustrol A. The final results of our study candidate erythrinassinate C for the development of a new drug against E. faecalis.

146 - CIPROFLOXACIN-LOADED MUCOSOMES AS NEW ANTIBACTERIAL APPROACH AGAINST STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA LUNG INFECTIONS IN VITRO.

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Ciprofloxacin-loaded mucosomes as new antibacterial approach against Staphylococcus aureus and Pseudomonas aeruginosa lung infections in vitro.

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1. Introduction. Bacterial infections are one of the most common causes of death in the world, mainly due to the rapid emergence of antibiotic-resistant bacteria that represent a relevant threat to public health. This challenging issue has motivated the scientific community to develop new alternative treatments to fight infection. Nanotechnologies provide outstanding tools as nanoweapons that can combat bacterial infections much more effectively than traditional antibacterial therapies. One promising approach involves mucosomes -mucin based nanoparticles- as multi-drug delivery platform.

2. Materials and Methods. Mucosomes were synthesized and loaded with ciprofloxacin. Dose dependance studies were performed in planktonic, biofilm and 3D cultures to evaluate the performance of drug-loaded mucosomes against Staphylococcus aureus and Pseudomonas aeruginosa, as well as to compare them to the activity of the free drug. The ability of mucosomes to interfere with the formation of biofilms was also studied in vitro through specific scanning electron microscope (SEM) and confocal microscope (CLSM) assays.

3. Results. No differences in treatment efficacy were noted in planktonic bacteria. Both in planktonic and 3D cultures, the activity of ciprofloxacin persists following encapsulation. Given the release

kinetics of ciprofloxacin embedded in mucosomes, it seems sense to assume that, under these circumstances, mucosomes increase the drug's activity. SEM and CLSM analyses demonstrated mucosomes' capacity to disrupt bacterial biofilm.

4.Discussion and conclusions. Mucosomes represent a promising tool in the treatment of pathogens colonizing the mucosal district of the organism. These results encourage the further exploration of the proposed versatile platforms for the development of novel targeted and stimuli-responsive antimicrobial nanoformulations for future application in personalized infection therapies.

147 - REPURPOSING HIGH-THROUGHPUT SCREENING IDENTIFIES UNCONVENTIONAL DRUGS WITH ANTIMICROBIAL AND ANTIBIOFILM POTENTIAL AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM CYSTIC FIBROSIS PATIENTS.

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Repurposing high-throughput screening identifies unconventional drugs with antimicrobial and antibiofilm potential against methicillin-resistant Staphylococcus aureus from cystic fibrosis patients.

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Introduction. In cystic fibrosis (CF) patients, chronic lung infections caused by methicillin-resistant Staphylococcus aureus (MRSA) exacerbate the clinical outcome and increase the decline in lung function. Antibiotic therapy is challenging due to the increasing acquired and biofilm-related antibiotic resistance, thus highlighting the need for new drugs. For the first time, we identified potential novel anti-S. aureus therapeutics by screening a drug repurposing library for antibacterial and antibiofilm activities.

Materials and Methods. The Drug Repurposing Compound Library (MedChemExpress), consisting of 3386 approved and clinical drugs with different therapeutic indications, was screened at 0.1 mM to identify “hit” compounds against S. aureus SA2, an MRSA strain causing long-term persistence in a CF patient. All assays were carried out in Trypticase Soy broth (TSB) under aerobic atmosphere. After 24 h-incubation at 37°C, the antibacterial activity was evaluated spectrophotometrically and by cell viable count, whereas a crystal violet assay evaluated the activity vs. biofilm formation and preformed biofilms. A potential “antibacterial hit” (a-BACT) was identified for growth reduction of ≥ 90% (vs. unexposed control), whereas a potential “antibiofilm hit” had to affect biofilm formation (a-BIOF/f) or disperse preformed biofilms (a-BIOF/p) ≥ 75% and had no antibacterial activity (≤ 10%).

Results. The high-throughput screening identified 308 (9.1%) a-BACT, 55 (1.6%) a-BIOF/f, and 1 (0.4%) a-BIOF/p drugs that have been developed for research areas other than infection/antibacterial, suggesting that they have the potential to be repurposed as antibacterial agents toward S. aureus. Hits were stratified according to the relative research areas as follows: cancer (a-BACT: 147; a-BIOF/f: 20; a-BIOF/p: 1); neurologic disease (a-BACT: 46; a-BIOF/f: 6); metabolic disease (a-BACT: 24; a-BIOF/f: 6); cardiovascular disease (a-BACT: 24; a-BIOF/f: 7); inflammation (a-BACT: 42; a-BIOF/f: 6); endocrinology (a-BACT: 15; a-BIOF/f: 3); and others (a-BACT: 10; a-BIOF/f: 7). The research area mainly represented was “cancer” both for a-BACT and a-BIOF/f ($p<0.0001$ and $p<0.01$, respectively, vs. other areas). Notably, 76 hits were 100% active vs. planktonic cells, whereas Flumatinib (mesylate) – a second-generation tyrosine kinase inhibitor used for acute lymphoblastic leukemia - was the only one active against both biofilm formation and preformed biofilm.

Discussion and Conclusions. Several hits revealed relevant antibacterial and antibiofilm activity and may represent progenitor scaffolds to develop new drugs for treating S. aureus infections in CF patients. In vitro and in vivo efficacy/toxicity studies are ongoing for further screening.

149 - IS AN INTRAOPERATIVE IODOPOVIDONE IRRIGATION ABLE TO REDUCE BACTERIAL CONTAMINATION IN THE SHOULDER ARTHROPLASTY?

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Is an intraoperative iodopovidone irrigation able to reduce bacterial contamination in the shoulder arthroplasty?

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Introduction. Shoulder arthroplasty has increased in popularity over recent years and it is the third most common joint-replacement surgery. Shoulder periprosthetic joint infection (PJI) is a rare but serious complication that influences patient outcomes and remains a common reason for surgical revision. PJIs could be caused by Staphylococcus aureus, coagulase-negative staphylococci (CoNS), including mainly S. epidermidis, and Cutibacterium acnes. Several strategies, during hip and knee arthroplasties, have been investigated with the aim of reducing PJIs intraoperatively, particularly the use of intraoperative iodopovidone irrigation. However, no data pertaining its use following shoulder arthroplasty are yet available. On these grounds, the aim of the present study is to assess if an intraoperative iodopovidone irrigation could be able to reduce microbial contamination in shoulder arthroplasty and to determine the bacterial isolates' virulence characteristics.

Patients and methods. Fifty-five patients undergoing reverse total shoulder arthroplasty (RTSA) were included in the study. At the end of RTSA surgery, three different specimens were collected from the surgical field – including two swabs from the glenosphere and humeral component and one periprosthetic tissue – thereafter, an irrigation with iodopovidone was performed and other three samples, from the same sites, were collected. After sonication, the specimens were cultured on various agar media suitable for the growth of aerobic and anaerobic bacteria and incubated.

Subsequently, bacteriological investigations were carried out, and the resulting predominant bacterial types were identified and characterized to evidence resistant or biofilm-producing bacterial strains.

Results. We preliminarily observed a reduction in both anaerobic and aerobic bacterial growth for the samples collected after the post-operative irrigation respect to the pre-operative ones, with a reduction of about 75% and 100%, respectively. C. acnes was the predominant anaerobic pathogen isolated whereas CoNS, mainly S. epidermidis and S. hominis, were the most representative aerobic strains. Gram-negative bacteria were also found, specifically Escherichia coli and Moraxella osloensis. Notably, only few isolates were resistant for the assayed antibiotics, and regarding staphylococci a low number of strong biofilm producer was highlighted.

Discussion and Conclusions. These preliminary results corroborate the efficacy of the intraoperative irrigation in reducing the bacterial load in the operatory field, and in turn it might contribute to prevent the risk of PJIs in shoulder arthroplasty. An appropriate prophylaxis therapy and a multifaceted microbiological approach could ensure a significant reduction in patient infection rate and a potential decrease in associated economic costs.

ORAL presentation

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153 - EXPLORING THE ANTIFUNGAL POTENTIAL OF THE ANTIDEPRESSANT VORTIOXETINE

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Exploring the antifungal potential of the antidepressant vortioxetine

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Introduction. Vortioxetine was approved in 2013 by the Food and Drug Administration for the treatment of adults with major depressive disorder. This molecule acts as an inhibitor of serotonin reuptake receptors and as an antagonist, agonist and partial agonist of multiple serotonin receptors. In a microbial community as complex and diverse as the intestinal ecosystem, it is logical to assume that some of the microbes would be able to metabolize the drugs consumed and that this will have a growth promoting effect or a growth inhibiting effect (antimicrobial) on microbes. This study explores the antifungal activity of the multimodal antidepressant vortioxetine against 60 isolates of Candida spp. (C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, and C. auris), aiming to assess its inhibitory effects on planktonic growth.

Materials and Methods. To define the antifungal efficacy of vortioxetine minimum inhibitory concentration (MIC90) and minimum fungicide concentration (MFC90) were determined by CLSI broth microdilution assay. The 24h growth and time kill curves of Candida spp. in the presence or absence of vortioxetine at 2MIC, MIC and MIC/2 were obtained by CFU/ml evaluation. Intracellular reactive oxygen species (ROS) generation under the exposure of vortioxetine was measured by applying DCFDA assay.

Results. MIC90 and MFC90 values of vortioxetine range between 8 µg/mL and 16 µg/mL for all selected Candida species (10 for each selected species) (Table 1).

Table 2

Species (no. of isolates)	MIC_{90} (µg/mL)	MFC_{90} (µg/mL)
C. albicans (10)	8	16
C. auris (10)	16	16
C. glabrata (10)	8	16
C. krusei (10)	8	16
C. parapsilosis (10)	16	16
C. tropicalis (10)	8	16

At vortioxetine concentrations equal to 2MIC for all species and MIC for C. auris and C. parapsilosis the number of CFU/mL decreased >4 log units (99.9% killing) by incubation for 24h (Figure 1).

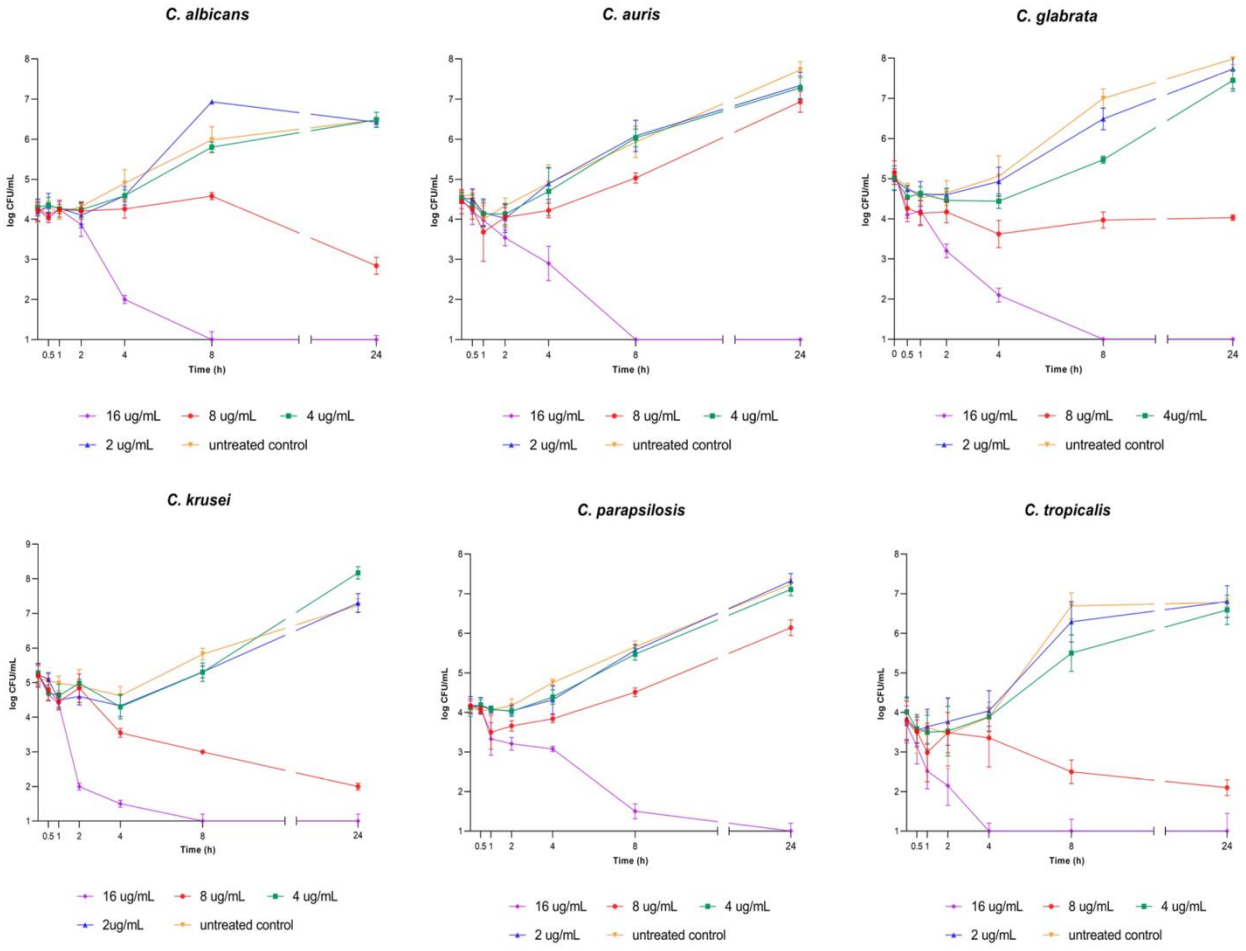

Figure 1

ROS generation by vortioxetine was examined after incubating 16, 8, 4 and 2 µg/mL of vortioxetine with the Candida cells. The cells exposed to vortioxetine recorded a considerable increase in the level of intracellular ROS when related to the untreated control samples. In addition, vortioxetine enhanced the production of ROS in a dose-dependent manner (**** = p<0,0005; *** = p < 0.001) (Figure 2).

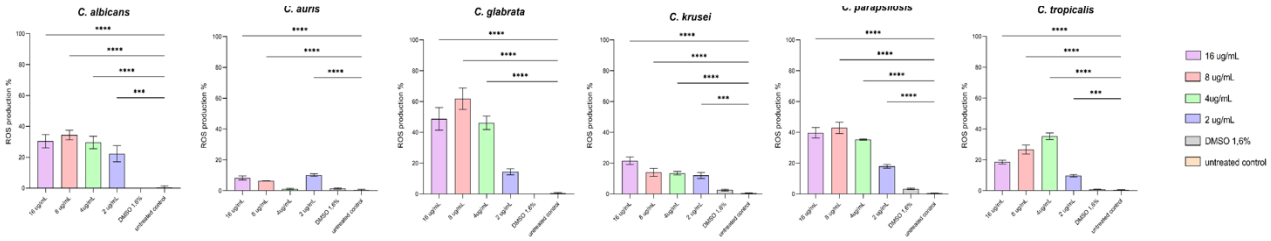

Figure 2

Discussion and conclusions. In this study the antifungal action of the antidepressant vortioxetine was demonstrated. This, along with other studies represents substantial evidence that antidepressants can affect the structure of the intestianal microbiome and that microbioma could affect the effectiveness of treatment.

158 - EXPLORING A NOVEL PHAGE-BASED APPROACH TO COMBAT MULTIDRUG RESISTANCE IN ACINETOBACTER BAUMANNII

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Exploring a novel phage-based approach to combat multidrug resistance in Acinetobacter baumannii

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Introduction: The dawn of the post-antibiotic era necessitates alternative strategies to fight infections. Bacteriophages (phages) have garnered interest due to their potential antimicrobial activity. Their advantages include high host specificity and co-evolution with bacteria. However, their narrow host range requires the isolation of specific phages for each bacterial strain. This study aims to discover and characterize novel phages targeting MDR Acinetobacter baumannii that can synergize with antibiotics and train an AI tool to detect phages in wastewater and that could be helpful in the choice of best phage targeting different bacterial isolates.

Methods: A multidrug-resistant (MDR) A. baumannii strain, resistant to cefiderocol and carbapenems, was cultured with two wastewater samples. Phages were isolated using a double-layer agar assay, visualized under transmission electron microscopy (TEM), and sequenced using long-read sequencing (ONT) to determine their families. Bioinformatics analysis predicted their life cycle. The host range of the isolated phages was evaluated by plaquing them on 12 A. baumannii strains. Additionally, the synergy between two phages and cefiderocol or meropenem was investigated using a checkerboard assay. Due to the novelty of the phages sequence, they were used as input to test the capability of the PhaMer AI-prediction tool to classify them as phage.

Results: Two distinct phages were isolated from each wastewater sample, with two different morphology identified as Siphoviridae and Podoviridae. Full genome sequencing confirmed their families, with sizes around 80kb (Siphoviridae) and 43kb (Podoviridae). All phages showed a narrow host range being able to infect only another A. baumannii strain with 99.74% genomic similarity to the enriching strain. Two Siphoviridae phages with lytic activity exhibited synergistic effect with cefiderocol (FIC index of 0.350 and 0.262), restoring susceptibility in one case (MIC of ≤2mg/L). No

synergistic activity was observed with meropenem. Despite not being deposited in any phage bank, the whole genomes were classified as phages by the PhaMer AI-prediction tool.

Discussion and conclusion: The newly isolated phages enhanced cefiderocol efficacy against MDR A. baumannii. Their discovery, coupled with genomic matching, suggests the potential for creating a biobank to expedite phage selection. Aligning novel phages with their host genomes could streamline the selection process. Although still not on clinical practice, several groups have reported clinical successes in treating infections caused by MDR bacteria with phage therapy, these findings could be important for a future implementation of phage therapy alone or in combination with antibiotics.

166 - BIOINSPIRED ORIENTED CALCIUM PHOSPHATE NANOCRYSTALS ARRAYS WITH BACTERICIDAL PROPERTIES AGAINST CARBAPENEMS-RESISTANT P.AERUGINOSA AND METHICILLIN-RESISTANT S.AUREUS

Damiano Squitieri *(1) - Lorenzo Degli Esposti (2) - Camilla Fusacchia (2) - Margherita Cacaci (1) - Riccardo Torelli (3) - Maurizio Sanguinetti (3) - Michele Iafisco (2) - Francesca Bugli (1)*

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Bioinspired oriented calcium phosphate nanocrystals arrays with bactericidal properties against carbapenems-resistant P. aeruginosa and methicillin-resistant S. aureus

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Introduction

Mitigating antimicrobial resistance requires evidence-based public health measures to prevent the projected O'Neill scenario by 2050. Action should go beyond creating new antibiotics and should focus on agents with resistance-avoidance bactericidal mechanisms. Neotibicen Canicularis, a cicada species, possesses a unique nanostructured surface on its wings, which is antimicrobial through a contact-based mechanism. This finding has prompt the development of synthetic bioinspired antibacterial nanostructured surfaces (ANS) which can counteract antibiotic resistance. Calcium phosphates (CaP) are bioactive materials with excellent biocompatibility, making them promising materials for developing ANS as coatings for orthopedic devices to prevent infections. This study aims to fabricate and characterize organized bioinspired CaP nanocrystals arrays for potential medical use.

Materials & Methods

CaP nanocrystals arrays were grown through a novel bottom-up biomineralization approach onto amorphous calcium phosphate (ACP) substrates. Tested substrates were ACP (ACP.GS) or citrate-stabilized ACP (Cit-ACP.GS). Cit-ACP.GS was further functionalized with Zn2+ ions through ionic exchange (Cit-ACP.GS-Zn4, not shown in figures) for antimicrobial enrichment. Flat control surfaces without nanostructuration ACP.H2O and Cit-ACP.H2O were prepared as references. Physico-chemical analysis were performed by FEG-SEM and Powder X-ray diffraction (PXRD). Tested bacterial strains were carbapenems-resistant P.aeruginosa and methicillin-resistant S.aureus. VERO fibroblast-like kidney cells were used for mammalian cell biocompatibility evaluation. Antimicrobial and biocompatibility assessments encompassed LIVE/DEAD bacterial and mammalian fluorescent assays, Scanning Electron Microscopy (SEM) imaging, and intracellular ROS quantification.

Results

The findings highlight the remarkable antimicrobial and biocompatible attributes of ANS, notably those grew on Cit-ACP substrates. Confocal and electron microscopy revealed a decrease in adherent viable bacterial cells on nanocrystals arrays, coupled with an elevated oxidative stress indicative of a contact-based bactericidal mechanism. Conversely, there was no discernible cytotoxicity in mammalian cells, confirming the complete absence of toxicity in CaP-derived materials and concurrently indicating a proliferative stimulus for the cells.

Discussion and Conclusions

Biomimetic ANS based on oriented CaP nanocrystals arrays showed important in vitro potential against antibiotic-resistant pathogens. ANS' contact-based mechanism of action and biocompatibility spectrum need to be further investigated for their potential orthopedic device manufacturing usage.

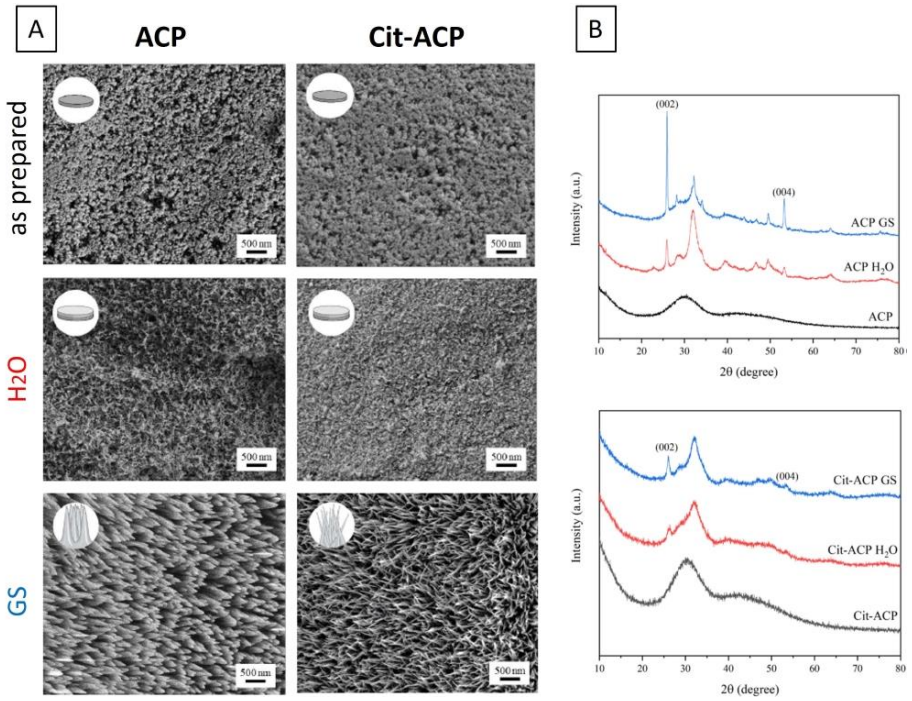

Figure 1- (A) FEG-SEM images of tested surfaces (B) PXRD analysis of tested surfaces

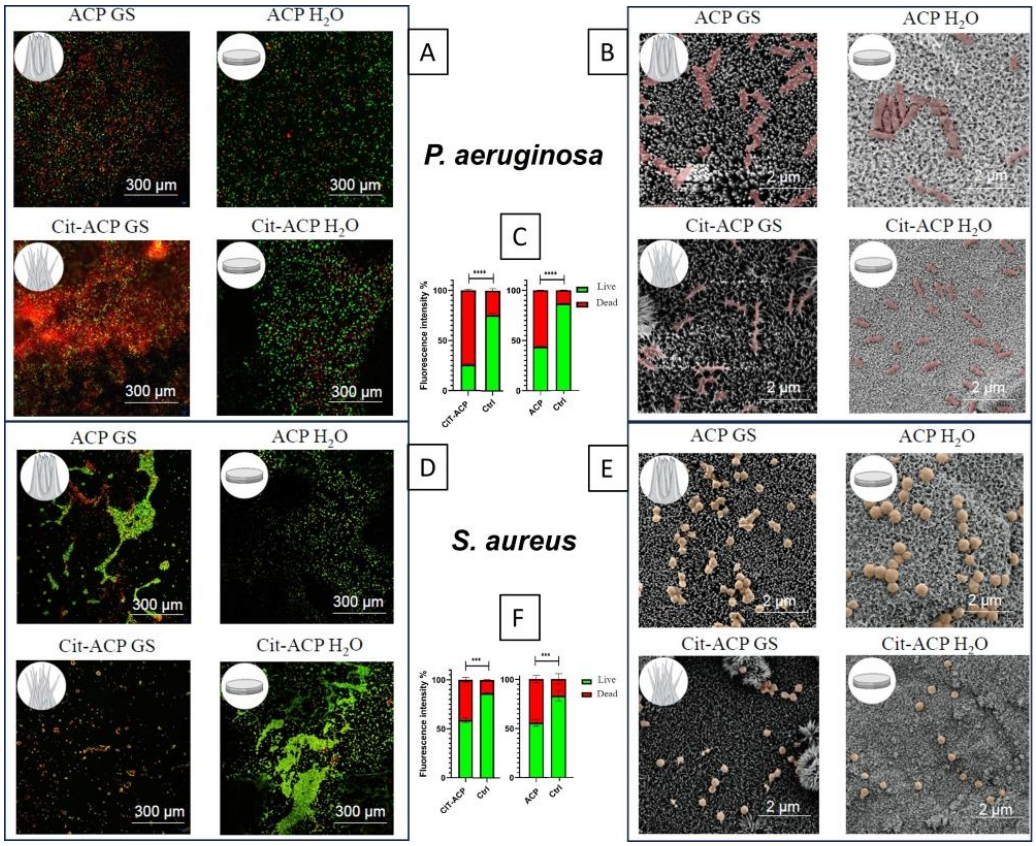

Figure 2 - (A,B,D,E)LIVE/DEAD and SEM images of P.aeruginosa and S.aureus(C,F)LIVE/DEAD fluorescence analysis

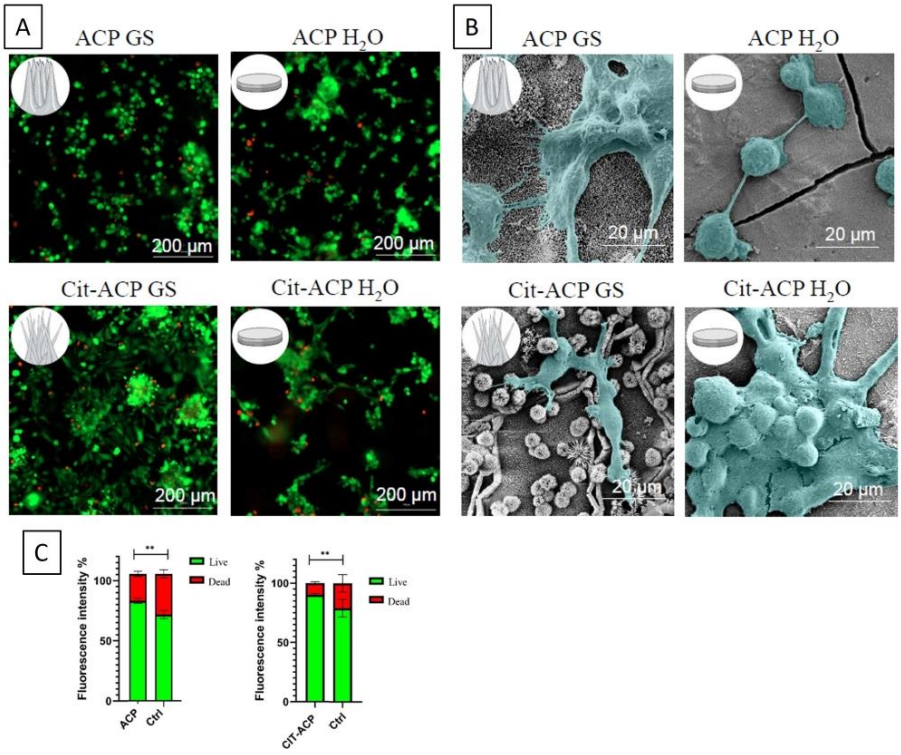

Figure 3 - (A,B) Mammalian LIVE/DEAD and SEM images of VERO cells (C) LIVE/DEAD fluorescence analysis

170 - INVESTIGATING THE ANTIVIRAL POTENTIAL OF HS-1 PEPTIDE AND ITS ALA-SCANNING ANALOGS DERIVED FROM AMPHIBIAN SKIN SECRETIONS

Bianca Maria Nastri *(1)* ***- Annalisa Chianese*** *(1)* ***- Maria Vittoria Morone*** *(1)* ***- Roberta Della Marca*** *(1)* ***- Carla Zannella*** *(1)* ***- Rosa Giugliano*** *(1)* ***- Alessandra Monti*** *(2)* ***- Carla Isernia*** *(3)* ***- Nunzianna Doti*** *(2)* ***- Anna De Filippis*** *(1)* ***- Massimiliano Galdiero*** *(1)*

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Investigating the Antiviral Potential of HS-1 Peptide and its Ala-Scanning Analogs Derived from Amphibian Skin Secretions

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Introduction. Viral infections represent a significant and growing threat to global public health. The limited efficacy of current antiviral therapies, which often target specific viruses, underscores the need for the discovery of broad-spectrum antiviral agents. Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs), have emerged as promising candidates for antiviral therapy due to their diverse activities against various pathogens. Amphibian skin secretions, a rich source of AMPs, represent an emerging class of therapeutic agents in several fields. In this study, we aimed to investigate the antiviral properties of HS-1 peptide, a frog-derived peptide from Hypsiobas semilineatus secretion, and its ala-scanning analogs. Materials and Methods. Peptides were synthesized using the solid-phase Fmoc chemistry method and subsequently purified through reversed-phase HPLC to ensure high purity. To explore the structure-activity relationship of HS-1 peptide, we employed ala-scanning mutagenesis, systematically substituting each residue with alanine to assess its impact on antiviral activity. Cytotoxicity assessments were conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The antiviral activity of HS-1 peptide and its analogs was evaluated against a diverse panel of viruses, including enveloped, naked, DNA, and RNA viruses, utilizing plaque assays and molecular tests. Results. Preincubation of HS-1 peptide with viral particles demonstrated significant antiviral activity, indicating its potential to disrupt

viral envelopes and interfere with extracellular phases of viral lifecycle, particularly viral attachment and entry. Ala-scanning mutagenesis demonstrated which was the critical residues essential for antiviral activity, with all of them exhibiting reduced cytotoxicity and some of them enhanced antiviral effects compared to the native peptide. Discussion and Conclusions. The results of this study underscore the promising potential of amphibian skin peptides, such as HS-1 and its analogs, as novel antiviral agents. The result highlights their utility as broad-spectrum antiviral therapeutics. Further elucidation of the specific mechanisms of action and viral targets of these peptides will be crucial for advancing their therapeutic development and combating viral infections effectively.

172 - GREEN-SYNTHESIZED SILVER NANOPARTICLES FROM ARCTOSTAPHYLOS UVA-URSI LEAF EXTRACT AS INNOVATIVE STRATEGY AGAINST SKIN INFECTIONS

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Green-synthesised silver nanoparticles from Arctostaphylos uva-ursi leaf extract as innovative strategy against skin infections

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Introduction. The therapeutical explorations of nanoparticles as antimicrobial and antiviral agents have gained more attention in recent years especially due to increasing incidences of antibiotic resistance. Nature-friendly nanoparticles from plants have been widely applied in the treatment of various microbial infections for their low toxicity. Here, we describe the synthesis of silver nanoparticles (AgNPs) in the presence of Arctostaphylos uva-ursi leaf extract and their potential as antibacterial and antiviral agents. A skin dressing was developed by incorporating AgNPs into a polysaccharide film as a promising strategy in the therapy of acne and oral herpes. Materials and Methods. A preliminary characterization of the AgNPs was conducted using nanosight, dynamic light scattering (DLS), zeta potential and transmission electron microscopy (TEM). Two different gellan/alginate (1.6:0.4 or 1.2:0.8) films were optimized as AgNPs vehicle and a Franz cell has been exploited to evaluate the silver skin permeation. The potential of AgNPs alone and conjugated with film was investigated against Staphylococcus epidermidis (S. epidermidis) and herpes simplex viruses type 1 and 2. The antimicrobial activity and time-killing assays were performed to evaluate the effects of AgNPs against planktonic bacteria. The influence of AgNPs on biofilm formation phases was also investigated by the crystal violet (CV) method. The cytotoxic activities of AgNPs were assessed on epithelial cells, using the 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT) test. The antiviral effect was analyzed via plaque reduction assays and confirmed by Western Blot and qPCR analyses. Results. The AgNPs minimum inhibitory concentration (MIC), exerting a bacteriostatic action on 90% of planktonic S. epidermidis (MIC90), was 2 x 107 NPs/mL and a 50% of inhibition (MIC50) was

recorded at 4 x 106 NPs/mL. AgNPs MIC90 and MIC50 concentrations were also able to disrupt the 56 and 34% of mature biofilm biomass. MTT test revealed that AgNPs had a very low toxicity on epithelial cell line, with a 70% of cell viability at the highest concentration tested. Moreover, AgNPs affected the early stages of viral infection, showing a complete inhibition up to 2 x 105 and 2 x 106 NPs/mL against HSV-1 and HSV-2, with an IC50 of 3 x 103 and 3 x 105 NPs/mL, respectively. Antiviral activity was verified by the reduction of expression of the gene UL27 and the corresponding envelope glycoprotein B in the presence of AgNPs. Discussion and Conclusions. The green-synthesized AgNPs displayed a potent antimicrobial activity directed against bacteria and viruses. They could represent a natural, ecofriendly, cheap, and safe method to produce an alternative antimicrobial strategy to combat skin infections.

176 - DECIPHERING THE SECONDARY RESISTOME OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) TO BETA-LACTAMS: A MULTI-OMICS APPROACH

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Deciphering the Secondary Resistome of Methicillin-Resistant Staphylococcus aureus (MRSA) to Beta-lactams: A Multi-Omics Approach

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Introduction: Antimicrobial resistance (AMR) poses a significant threat to global health, affecting both human and animal settings. Methicillin-resistant Staphylococcus aureus (MRSA), a prevalent multidrug-resistant bacterium, is a high-priority pathogen for new antibiotic development. This study investigates a potential solution to AMR: making existing antimicrobials effective again. We aim to understand the secondary resistome of MRSA, which plays a key role in its resistance to beta-lactam antibiotics.

Materials and Methods: A wide-genome screening of a highly saturated MRSA transposon mutant library was conducted using Transposon-Directed Insertion site Sequencing (TraDIS), following exposure to subinhibitory concentrations of oxacillin and cefazolin. The resulting reads were analyzed using the BioTradis pipeline. Subsequently, label-free quantitative proteomics was conducted on the antibiotic-exposed cultures. The mass spectrometer data was processed using Proteome Discoverer, and Perseus v1.6 was used for statistical analysis.

Results: Our comprehensive multi-omics dataset, which integrates proteomics and TraDIS, identified a set of conditionally essential genes/proteins and revealed the metabolic dynamics of MRSA's resistance to beta-lactams. Our findings suggest that purine metabolism, mediated by second messengers c-di-AMP and (p)ppGpp in conjunction with the three-component system vraRST, plays a pivotal role in resistance by activating the cell wall stress stimulon. We also identified a set of genes involved in purine metabolism, DNA repair, and the spx degradation system. The inactivation of these genes enhances the bacterium's resistance phenotype. Our proteomics analysis, conducted post-antibiotic exposure, revealed significant changes in bacterial proteome expression, following stringent response pattern mediated by (p)ppGpp.

Conclusion: This study provides novel insights into the secondary resistome and metabolic changes associated with beta-lactam resistance in MRSA. By integrating large-scale transposon mutagenesis and proteomics, we present valuable data identifying potential targets for adjuvant drugs that could be synergistically employed with beta-lactams. We also provide information on mutations that could potentiate antimicrobial resistance, which could be used to predict high resistance to beta-lactams.

179 - SILVER NANOPARTICLES PRODUCED BY PULSED LASER ABLATION IN LIQUID (PLAL) AGAINST CORONAVIRUS INFECTIONS.

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Silver nanoparticles produced by Pulsed Laser Ablation in Liquid (PLAL) against coronavirus infections.

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Introduction. The emergence and re-emergence of viral infections is a serious problem for global public health. Vaccination remains the most effective tool to control respiratory infections of viral origin, such as influenza, bronchiolitis, pneumonia, and the current COVID-19. However, there are still numerous viruses against which we have no preventive or therapeutic approaches. In this context, the search for new antiviral agents and innovative antiviral preventive strategies has become extremely important and the topic of intense scientific study. Nanomaterials are of great interest because they have a lot of potential to create new and innovative products across many areas. In this study, we examined the antiviral activity of silver nanoparticles (AgNPs) synthesized through a physical strategy that uses laser ablation in liquid (PLAL). Materials and Methods. AgNPs were characterized by different chemical/physical techniques including Total X-Ray Fluorescence (TXRF), Zeta Potential, UV-vis spectrum, and Transmission Electron Microscopy (TEM) analysis. The antiviral potential was investigated against some members of the Coronaviridae family, both through plaque reduction assays and molecular test. Results. Through Nanoparticles Tracking analysis (NTa), AgNPs produced in liquid (PBS) were counted. Then, we assessed their cytotoxicity on Vero-76 cells in a range of concentration from 4.6 x 107 to 1.8 x 105 by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Starting from non-cytotoxic concentrations, we examined the antiviral potential

against the alphacoronavirus HCoV-229E and the betacoronaviruses SARS-CoV-2 and HCoV-OC43. For all three viral models considered, our system resulted active by interacting directly with the envelope at an early stage of infection. This result was confirmed by Real-Time PCR, by evaluating the expression of the genes involved in the viral infection. Discussion and conclusions. The multiplicity of actions of AgNPs obtained through PLAL suggests that they could represent promising broad-spectrum antimicrobial agents. However, other studies will be necessary to deepen their mechanism of action.

181 - ATTIVITÀ ANTIBATTERICA DI ESTRATTI DI SCARTI DI LUPPOLO (HUMULUS LUPULUS) NEI CONFRONTI DEL FITOPATOGENO ERWINIA AMYLOVORA

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Attività antibatterica di estratti di scarti di Luppolo (Humulus lupulus) nei confronti del fitopatogeno Erwinia amylovora

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Introduzione: Erwinia amylovora (EA) è un fitopatogeno da quarantena, agente causale del colpo di fuoco batterico, malattia che colpisce le Rosacee [1]. La resistenza agli antibiotici ed al rame ha portato a porre l'attenzione su fitofarmaci di origine naturale. In questo studio è stata testata su tre ceppi batterici di EA, isolati da meli dal Dipartimento Fitosanitario Regionale dell'Emilia-Romagna, l'attività antimicrobica di diversi Estratti di luppolo (HOPE): metanolico (MeHOPE), idroalcolico 80:20 (HaHOPE) ed idroalcolico 70:30 (HaaHOPE). In seguito, è stato studiato il meccanismo con cui gli estratti possono influenzare la replicazione e lo sviluppo di fattori di virulenza quali la sintesi di amilovoro, la formazione di biofilm e la motilità [2].

Materiali e metodi: La MIC e l'MBC sono state valutate attraverso il metodo in sospensione e il metodo della conta microbica in piastra, utilizzando un inoculo alla concentrazione di 106CFU/ml a cui sono stati aggiunti gli estratti a diverse concentrazioni. Il test sulla motilità è stato svolto inoculando 105CFU/ml di EA al centro di piastre di soft agar con concentrazioni dei diversi HOPE minori della MIC (subMIC) precedentemente determinata, e misurando lo spostamento dopo 2/6 giorni di incubazione. Per l'inibizione del biofilm e per la riduzione di amilovoro sono stati inoculati 106CFU/ml di EA con le concentrazioni subMIC di MOE. Per il primo test le piastre sono state incubate a 26°C per 6 giorni e il biofilm evidenziato con cristalvioletto, per la riduzione dell'amilovoro sono stati incubati a 4°C per 4 giorni in terreno MBMA.

Risultati: Gli estratti idroalcolici dimostrano attività batteriostatica alla concentrazione di 2 mg/ml e portano ad una riduzione della formazione di biofilm dell'80%. Per l'inibizione della motilità mostrano una riduzione del 38% e 43% dopo 6 giorni ed una diminuzione della sintesi di amilovoro del'53% e 71% rispettivamente per HaHOPE e HaaHOPE,

Discussione e conclusioni: L'azione antibatterica degli estratti si imputa alla presenza di alte quantità di composti polifenolici, confermata tramite HPLC. Questi sono in grado di alterare la permeabilità della membrana batterica e di conseguenza la motilità, comportando sia un rallentamento della

sintesi di ATP sia una minor selettività nei confronti dei composti del fitocomplesso che possono così entrare nel citoplasma batterico ed inibire enzimi coinvolti nella replicazione e nel quorum sensing [3].

La buona efficacia, l'ecocompatibilità ed il basso costo rendono tali estratti dei potenziali mezzi di controllo del colpo di fuoco batterico, assicurando una miglior efficacia se usati sotto forma di "cocktail" o come "enhancer" dell'attività antimicrobica di altri prodotti.

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182 - ANTIBIOFILM EFFECT OF RESVERATROL ON STAPHYLOCOCCUS AUREUS CLINICAL STRAINS

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Antibiofilm effect of resveratrol on Staphylococcus aureus clinical strains

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Introduction: Biofilm-associated chronic infections caused by Staphylococcus aureus often lead to significant increase in morbidity and mortality. This problem has elicit a lot of researches to develop new therapeutic strategies against S. aureus infections. On these assumptions we aimed to study the antimicrobial action of resveratrol (3, 4', 5 trihydroxystilbene), a compound present in grape skin and wine, and also known to exhibit multi-spectrum therapeutic applications. In order to increase its efficiency we conjugated resveratrol (RSV) with nanostructured lipid vectors (NLC) and evaluated the antibiofilm action of RSV and RSV-NLC on S. aureus clinical strains

Materials and methods: Fifty S. aureus clinical strains isolated of from different hospitals were examined. The biofilm formation was measured by Crystal Violet (CV) assay. The strains were grown in MH medium and then the antibiofilm action of RSV and RSV-NLC (16µg/ml) was established in flat-bottom 96-well microtiter plate wells. Specifically, the strains were incubated at 37 °C for 24 h with the molecules to be assayed, washed with PBS, stained with CV(0.5%) and finally the optical density (OD) of each wall was measured at 570 nm using spectrophotometer. Treated and untreated strains were repeated in triplicate

Results: The clinical isolates of S. aureus examined (50) were 56% (28) high biofilm producers (strongly adherent, SA), 30% (15) medium biofilm producer (moderately adherent) and 14% (7) low biofilm producers (weak adherent). The 28 SA strains after treatment with RSV, showed the following results: 20% (10) remained strongly adherent, 32% (16) showed a reduction in biofilm production and became moderately adherent, and only 0,5% (1) was downgraded to weakly adherent. When the same 28 strains were treated with RSV-NLC, in five cases did not reduce biofilm production, in 13 (46%) downgraded to moderately adherent, the remaining of the isolates showed a significant reduction in biofilm by classifying themselves as weak adherent

Discussion and conclusions: In our study to evaluate the action of tran-resveratrol on gram-positive strains, biofilm assay was performed. The data obtained showed a good action of the polyphenol on S. aureus strains. After the treatment, many strains undergone to significant reduction in biofilm production. Treatment with NLC-conjugated trans-resveratrol gave similar results as free trans-resveratrol. On the basis of these data we believe that RSV-NLC could be used in combined therapy to avoid the onset of MDR strains and also any side effects of drugs as well when used at high dose

185 - NOVEL ANTIBACTERIAL NANOCONSTRUCTS BASED ON CARBON DOTS OR CALIXARENE DERIVATIVES INCORPORATING NATURAL BIOACTIVE COMPOUNDS AND CIPROFLOXACIN FOR COMBINED EFFECT.

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Novel antibacterial nanoconstructs based on carbon dots or calixarene derivatives incorporating natural bioactive compounds and ciprofloxacin for combined effect.

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Introduction. An appealing strategy to counteract the increasing antimicrobial resistance is represented by combinations of natural and conventional antimicrobial molecules and their entrapment into nanocarriers for simultaneous delivery. The present study aims to develop novel antimicrobial drug delivery systems by co-loading natural hydrophobic compounds, namely carvacrol (CAR) and curcumin (CUR), and conventional ciprofloxacin (CPX) in nanocarriers for multidrug treatment of antibiotic resistant infections. Materials and Methods. Two classes of nanocarriers were prepared and their physicochemical properties were characterized. Specifically, a nanocarrier composed of a carbonaceous core (carbon-dot) covered by β-cyclodextrin units (Cdots-βCD) and nanocarriers generated by the self-assembling of amphiphilic calixarene derivatives were studied. The drug loading capacity percentage of the nanocarriers was calculated. The carrier-drugs interactions were investigated by molecular dynamics simulations, the HOMO energy (nπ* transition) for Cdots-βCD/CPX complex was calculated, and the UV-Vis spectra simulated. The in vitro antibacterial and antibiofilm activities were investigated by broth microdilution and biofilm biomass measurement against methicillin resistant Staphylococcus aureus, ESBLs producing Escherichia coli, Acinetobacter baumannii, and VIM-2 producing Pseudomonas aeruginosa. The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Results. The carrier-drug nanoconstructs showed good dispersibility in aqueous solution, high chemical stability, biocompatibility and optical properties including luminescence and photothermal effect at 405 nm. The Cdots-βCD/CAR nanosystem and the micellar sulfonate-calixarene co-loading CUR and CPX demonstrated antibacterial efficacy comparable or even better than that of the individual bioactive compounds. Notably, Cdots-βCD/CAR exhibited MIC values ranging from 17.5 to 70 μg/mL and more than 50% inhibition of S. aureus biofilm formation at sub-MIC concentrations. Interestingly, the sulfonate-calixarene/CPX/CUR nanoconstruct was found to possess antioxidant activity. Discussion and Conclusions. The designed nanocarriers

appear as promising candidates for improving solubility, stability, and bioavailability of the selected bioactive compounds. The presence of multiple sites of complexation also allows their co-delivery for additive or synergistic effects. Our findings suggest that the novel nanoconstructs could be favorable delivery systems for the treatments of antibiotic resistant infections.

Acknowledgments

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190 - EXPLORING THE ANTIBACTERIAL AND BIOCOMPATIBILITY PROPERTIES OF BIODEGRADABLE MAGNESIUM ALLOY FOR ORTHOPEDIC PROSTHETIC FIELD

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Nuovi approcci antimicrobici

Exploring the antibacterial and biocompatibility properties of biodegradable magnesium alloy for orthopedic prosthetic field

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Introduction

Orthopedic diseases pose a significant challenge in the medical field, often requiring innovative solutions to address the unique patient needs. Magnesium-based alloys (i.e., WE43) have emerged as a promising material for use in orthopedic applications. In particular, WE43 has a density similar to that of human bone, which can help to reduce stress shielding, a common issue with traditional metallic implants. Moreover, magnesium alloys possess the ability to degrade over time, allowing the implant to be gradually replaced by the bone tissue, a process known as biodegradation. This property can potentially eliminate the need for additional surgical procedures. However, corrosion and rapid degradation limit their use due to implant functionality loss before bone union. One of the crucial aspects of orthopedic implants is to resist bacterial colonization, which can lead to severe complications compromising the procedure's success. Recent studies have suggested that the WE43 alloy may possess intrinsic antibacterial properties. The corrosion behavior of WE43 has been found to generate a localized pH increase, which can create an inhospitable environment for certain bacterial

species. This antibacterial effect can help to reduce the risk of post-surgical infections, a common concern in prosthetic orthopedics.

Materials and Methods

Magnesium alloy (WE43) probes were treated with an electrolyte containing Na_2SiO_3, NaOH and Na_3PO_4 (PEO-WE43) to reduce the degradation when in contact with biological fluids. Then the probes antibacterial properties were tested by disk diffusion against Pseudomonas aeruginosa as a model for prosthetic infections. Finally, the direct and indirect cytotoxicity was tested (24h exposure time) on lymphoblastic (CEM) and epithelial (VERO) cells, respectively.

Results

Our data clearly indicate a lower degradation of PEO-WE43 compared to WE43. Despite this, the antimicrobial effect was evident and similar for both WE43 and PEO-WE43, whose degradation does not exhibit any indirect cytotoxicity on epithelial cells. However, the direct exposition to both probes blocked the proliferation of CEM cells.

Discussion and Conclusions

This work shows some of the WE43 potential against bacterial infections in orthopedic implants. The antibacterial activity against P. aeruginosa is more likely attributable to the probe itself than the product released during the degradation. In fact, the electrochemical coating does not influence the antibacterial activity. However, additional research on the long-term biodegradation of the modified material is mandatory. Overall, physico-chemical and antibacterial features, together with the preliminary biocompatibility results, strongly encourage further study of this alloy and its use in the prosthetic field.

194 - DIFFERENCES IN MICROBIAL COLONIZATION, CLINICAL SCORES AND ANTIBIOTIC-RESISTANCE CARRIAGE BETWEEN SURGERY AND MONOCLONAL TREATMENT IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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DIFFERENCES IN MICROBIAL COLONIZATION, CLINICAL SCORES AND ANTIBIOTIC-RESISTANCE CARRIAGE BETWEEN SURGERY AND MONOCLONAL TREATMENT IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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Chronic rhinosinusitis with nasal polyps (CRSwNP) is a multifactorial condition, characterized by persistent inflammation of the paranasal sinuses and aggravated by nasal microbiome alterations. The usual treatment of endoscopic surgery is invasive and can lead to recurrent CRSwNP, so alternatives like monoclonal antibodies inhibiting type-2 inflammation (Dupilumab) can be promising for restorative therapy. For the first time, this prospective study compared the microbial profiles, clinical outcomes, and antibiotic-resistance carriage in patients with CRSwNP undergoing surgery or monoclonal treatment.

44 patients with CRSwNP were divided in two randomized groups: 22 Dupilumab, 22 endoscopic surgery. Clinical assessments, like SNOT-22 and SS-IT scores, and microbiota composition of nasal swabs were assessed at treatment-naïve baseline and follow-up after 6 months. Isolated strains were identified by PCR and sequencing, and antibiotic resistance was measured with the E-test method.

At baseline, both groups showed similar clinical scores and bacterial profiles, but CRSwNP-relevant species like Pseudomonas aeruginosa and Staphylococcus aureus were more abundant in the monoclonal group. At follow-up, with Dupilumab P. aeruginosa was eradicated (22% to 0%), while Staphylococcus epidermidis and S. aureus increased in the surgery group (28% to 77%, 23% to 50% respectively). Moreover, S. aureus and P. aeruginosa were associated with the worst outcomes with higher SNOT-22 scores, while S. epidermidis exhibited lower SNOT-22 and higher SS-I scores. The best outcomes were found when S. epidermidis and Dupilumab treatment were paired. For antibiotic resistance profiles, 25.3% of S. epidermidis and 19.3% S. aureus were classified as MDR (resistant to cefoxitin, gentamycin, clindamycin, erythromycin), whereas 28.6% of P. aeruginosa and 20% Enterobacterales were MDR (resistant to piperacillin, ceftazidime, aztreonam).

This was the first clinical study to compare clinical scores, microbial colonization, and antibiotic-resistance in CRSwNP with two different treatments. Interestingly, results show a better response to Dupilumab, corroborating the use of new targeted therapies for this condition.

199 - TIN-BASED COMPOUNDS AS NEW POTENTIAL AGENTS TOWARDS HTLV-1

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Tin-based compounds as new potential agents towards HTLV-1

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Introduction. Human T-Lymphotropic virus type 1 (HTLV-1) is the causative agent of two distinct pathologies, adult T-cell Leukemia/lymphoma (ATL), and tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM), and infection is characterized by a life-long latency. Currently there are no effective therapies or vaccines to counteract or prevent infection or diseases caused by HTLV-1. Metal-derived platinum complexes are amply utilized for the treatment of solid tumors. However, the developed drugs suffer from systemic toxicity and drug resistance. Recently tin derivatives have been investigated as promising anti-cancer drug candidates. No information is available on effects of tin-based compounds in viral infections Methods. In the presented study the effects of a newly synthetized tin-based compound, tributyltin 2,2,2-trifluoroacetate (TBT), on cellular and virological parameters in immortalized lymphocytes generated by HTLV-1 infection in vitro and rendered or not progressively independent from interleukin-2 as a growth factor or the C91/PL HTLV-1 transformed cell line, have been evaluated. As cellular parameters, cell metabolic activity, and apoptotic-like regulated cell death (RCD) in presence or in absence of RCD and autophagy inhibitors, were assessed. Moreover, molecular studies through real-time PCR and western blot have been performed to reveal possible targets of TBT and its effects on virus expression. Results. Twenty four hours treatment induced a dose dependent, differential inhibition of proliferation and cytotoxicity in the cell models used. A dose-dependent, apoptotic-like, but not-well-identified, form of RCD, in a dose/cell-type-dependent fashion was noticed. Particularly, experiments with specific inhibitors suggest that autophagic, pyropoptotic and/or necroptotic responses are involved in the occurring phenomenon, mostly in IL-2-dependent (IL-2D) and IL-2-independent (IL-2IND) cells. Inhibition of specific viral genes expression, especially Tax and HBZ in IL-2IND cells, following TBT treatment, was also detected. Discussion and Conclusions. Taken together, obtained data show for the first time that tin-based compounds can induce typical and potent effects in HTLV-1 infected cells, quite different from those induced by cis-platinum, and even if utilized at low concentrations for short time. It is notable the

inhibitory effect on viral HBZ gene expression in IL-2IND cells, i.e. in cells mimicking the HTLV-1-driven transformation phase. Further studies are necessary to reveal the potentiality of tin-based compounds in HTLV-1 infection. Funding. This work was supported by PRIN 2022 grant number W97H54_003 “Founded by European Union - Next Generation EU”.

200 - PHOSPHATIDYLSERINE LIPOSOMES AS A THERAPEUTIC STRATEGY FOR THE MANAGEMENT OF MYCOBACTERIUM ABSCESSUS INFECTION IN CYSTIC FIBROSIS PATIENTS

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Phosphatidylserine liposomes as a therapeutic strategy for the management of Mycobacterium abscessus infection in Cystic Fibrosis patients

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Introduction: Mycobacterium abscessus (Mab) is an opportunistic nontuberculous mycobacterium, intrinsically resistant to a wide range of antibiotics, responsible of difficult-to-treat pulmonary infections in vulnerable patients, like Cystic Fibrosis (CF) ones. Impaired macrophage function in CF patients plays a pivotal role in defective bacterial killing. Despite cystic fibrosis conductance regulator (CFTR) modulators, as Kaftrio (Kaf), are being widely used to improve CF patient quality of life and clinical outcome, there still is a percentage of patients whose CFTR mutations are incompatible for such pharmacological treatment. In this study, we have analyzed the in vitro therapeutic value of phosphatidylserine liposomes (PS-L) on Mab infected macrophages from i) healthy donors (HD) with pharmacologically inhibited CFTR, and ii) CF patients undergoing Kaf therapeutic regimen or not.

Materials and methods: Monocyte-derived macrophages (MDMs) from HD were treated or not with the pharmacologically inhibitor of CFTR (INH172) in order to obtain a CF-like phenotype. Thereafter, cells were in vitro infected with Mab and then stimulated or not with PS-L. Liposomes efficacy was assessed in terms of i) intracellular mycobacterial viability by CFU assay in the presence of absence of Peg-catalase (Reactive oxygen species (ROS) inhibitor) and Concanamycin-A (Phagolysosome acidification inhibitor), and ii) inflammatory response, evaluating NF-kB activation and TNF-alfa production by ELISA. Moreover, MDM from CF patients receiving or not Kaf therapeutic regimen, were in vitro infected with Mab and then stimulated or not with PS-L and/or Kaf. Treatment efficacy was evaluated in terms of Mab intracellular killing by CFU assay.

Results: Results show that PS-L treatment induces a significant phagosome acidification-dependent and ROS-mediated intracellular killing of Mab in MDM from HD, irrespective of CFTR inhibition. Additionally, PS-L stimulation resulted in a significant drop in NF-kB activation and TNF-alfa production. Finally, PS-L treatment enhances intracellular killing of Mab in macrophages from CF patients, particularly in those who are not undergoing Kaf therapeutic regimen and are completely ineligible for such pharmacological treatment.

Discussion and conclusions: These findings represent the proof of concept supporting a future development of a PS-L based host-directed therapeutic strategy, capable of simultaneously reducing

both potentially pathogenic pro-inflammatory response, and intracellular Mab viability in CF patients. In this context, PS-L may represent a possible additional therapeutic strategy for those patients who are not receiving Kaf treatment and who can't benefit from the improved antimicrobial response possibly deriving from such pharmacological therapy.

206 - EFFICACY AND SAFETY OF FORMULATIONS BASED ON ESSENTIAL OIL AGAINST CO-INFECTIONS OF HERPES SIMPLEX-1 AND CANDIDA ALBICANS

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Efficacy and safety of formulations based on essential oil against co-infections of Herpes Simplex-1 and Candida albicans

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Background and aim. Oral coinfections involving HSV and Candida albicans are common and can potentially interact and exacerbate each other. Starting from a careful bibliographical investigation, this study aimed to examine the effectiveness of some EOs, and their commercial formulations, both against C. albicans and HSV-1, identifying not only their antimicrobial activity but also their anti-inflammatory and allergenic potential.

Experimental procedure. Preliminary bibliographic research was done about the efficacy of EOs against HSV-1. Subsequently, broth micro-dilution test was used to test the activity of 14 EOs, an EOs commercial formulation (LA), and a homemade one (MIX) against three fungal strains. The quality of LA, MIX and single EOs was assessed by SPME sampling coupled with GC-MS analysis. To assess the allergenic activity of MIX, LA, and single EOs a Basophil Activation Test (BAT) was standardized. ELISA tests were done to evaluate the anti-inflammatory activity of both formulations.

Key results. The bibliographic research highlighted 7 EOs active against HSV-1. Four EOs showed an interesting antifungal activity and, in compliance with IFRA regulations, were used to formulate a mixture (MIX) to be compared against a commercial one (LA) made with 6% of EOs. Formulations were active against HSV-1, able to modulate the expression of cytokines TNF-α and IL-1β, and showed no

allergenic potential. In particular, MIX strengthens target cells and inhibits viral infection, while LA also inhibits intracellular replication.

Conclusions. The results support the use of EO-based formulations for the treatment of the increasingly common co-infections of HSV-1 and C. albicans because they are effective and reasonably safe.

210 - A FILM-FORMING SPRAY AS A NEW DELIVERY SYSTEM TO ENHANCE THE ACTIVITY OF COLISTIN-ENCAPSULATED NANOPARTICLES AGAINST MDR GRAM NEGATIVE BACTERIA

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A film-forming spray as a new delivery system to enhance the activity of colistin-encapsulated nanoparticles against MDR Gram negative bacteria

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Introduction: Evolution of resistance to last-resort antibiotics, such as colistin (Col), causes a relevant problem in the treatment of different nosocomial infections. In particular, a high susceptibility to infections by Multidrug-resistant (MDR) bacterial strains is found in chronic and post-burn wounds, making the treatment a growing challenge. Nanocarriers have been proposed for antibiotic delivery as a promising strategy to counteract infections increasing drug efficacy and penetration. The study aimed to develop a topical film-forming spray formulation containing colistin-loaded albumin nanoparticles (Col/haNPs-FFS) for the treatment of wound infections. Materials and methods: Blank and Col-loaded chitosan-coated human albumin nanoparticles (haNPs) were prepared with a purposely tuned double-emulsion method. The FFS was optimized using different amounts of Plastoid® B, ethyl acetate and PEG 400. Plastoid® B was used as the film-forming polymer, ethyl acetate to obtain a rapid film formation, and PEG 400 was used as plasticizer to enhance the volume of spray and the surface covered by the formulation after spray. The formulation was evaluated measuring pH, viscosity, uniformity of drug content, drying time, thickness of the film, spray angle and occlusion potential. Moreover, the formulation was evaluated for antimicrobial activity by time-kill assays as well as for biocompatibility by MTT assays using both in vitro and ex vivo systems. Results: The optimized Col/haNPs-FFS containing Plastoid® B (18% p/v), ethyl acetate (30% v/v), Col/haNPs (25% v/v), distilled water (25% v/v), PEG 400 (0,5% v/v) is easily sprayable thanks to its low viscosity (1.30 mPas) showing a 1.03° spray angle and 60s drying time. It rapidly forms a thin film (0.010 mm) that resulted non-occlusive (Occlusivity factor F=7.19 ± 1.97 %). The Col/haNPs-FFS shows good spray uniformity (Spray weight=132.844 mg ± 1.237). No cytotoxicity on epithelial cells and human skin demonstrated the safety of the formulation. The Col/haNPs-FFS shows a high antibacterial potency, reducing the number of MDR Col R and S Acinetobacter baumannii, respect to free Col. Discussion and Conclusions: Soft and skin tissue infections, caused by MDR GNB, are becoming

increasingly prevalent and constitute a global problem because they are difficult to treat. Our findings suggest that the topical FFS could be a valid approach for managing of burn and trauma-related injuries obtaining a colistin sustained release at the infection site.

248 - ENHANCED ANTIBACTERIAL ACTIVITY OF GRAPHENE QUANTUM DOTS THROUGH BLUE LIGHT STIMULATION

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Enhanced Antibacterial Activity of Graphene Quantum Dots through Blue Light Stimulation

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Introduction. Graphene Quantum Dots (GQDs), characterized by a 2D transverse dimension of less than 100 nm, possess remarkable chemical, physical, and biological properties. With their oxygen and hydrogen functional groups, they offer potential in antimicrobial photodynamic therapy (aPDT), a strategy to combat antibiotic resistance, especially against multidrug-resistant (MDR) bacteria.

Materials and Methods. GQDs, including Blue Luminescent (L-GQDs), Aminated (NH2-GQDs), and Carboxylated (COOH-GQDs) variants, were tested for antimicrobial activity against E. coli, in association with blue light irradiation (490 nm) for 15, 30 and 60 minutes by assessing colony forming units (CFUs) count. Additionally, GQDs were evaluated for cytotoxicity on human colorectal adenocarcinoma cells (Caco-2), utilizing MTS assay to assess metabolic activity. Direct activity of GQDs was also evaluated using Scanning Electron Microscopy (SEM). Finally, GQDs were tested for their efficacy in treating infected cells.

Results. Blue light stimulation of GQDs enhances their antibacterial activity against E. coli. Differential functionalized GQDs were administered to E. coli suspensions, followed by blue light irradiation for different durations. Enhanced antibacterial efficacy of GQDs with blue light stimulation, particularly notable after 60 minutes, was observed. A slight antimicrobial activity has been detected also after 24 hours of E. coli incubation. SEM analysis revealed no significant morphological changes in treated bacteria. Otherwise, MTS assay revealed minimal changes in bacterial metabolic activity with GQD

treatments alone, but significant decreases were observed after blue light exposure, suggesting a crucial role of GQDs in modulating bacterial metabolism under light stimulation. Once it was confirmed that GQDs didn't affect eukaryotic cells, an in vitro E. coli infection model on Caco-2 cells showed no reduction in microbial burden with GQDs, regardless of blue light stimulation.

Discussion and conclusions. Blue light stimulation enhances GQDs' antimicrobial efficacy against E. coli, suggesting potential in combating antibiotic resistance. GQDs exhibit minimal impact on bacterial morphology but significantly modulate bacterial metabolism under light stimulation. However, GQDs effectiveness in reducing microbial burden in a cellular infection model needs further research to elucidate their action during infection. Nevertheless, their synergistic action with antibiotics needs to be further investigated as an avenue for future antimicrobial strategies.

276 - ANTIMICROBIAL POTENTIAL EVALUATION OF LACTIC ACID BACTERIA ISOLATED FROM HUMAN BREAST MILK FOR BURN WOUND INFECTION MANAGEMENT IN THE IN VIVO ALTERNATIVE PRECLINICAL MODEL GALLERIA MELLONELLA

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Antimicrobial potential evaluation of Lactic Acid Bacteria isolated from human breast milk for burn wound infection management in the in vivo alternative preclinical model Galleria mellonella

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Introduction. Burn wound infection is a significant clinical complication that can lead to impaired healing and even mortality. Given the increase in antibiotic resistance, more and more attention is being paid to the role of bacteria in preventing or treating infection, especially Lactic Acid Bacteria (LAB). The latter have attracted attention due to their antimicrobial potential and immune-modulating properties. However, the potential implication of employing live bacteria on complex wounds such as burn ones is a pivotal point. Thus, in vivo models are pivotal in evaluating new therapeutic approaches. This study evaluated the safety profile and antimicrobial activity of LAB isolated from human breast milk on an alternative preclinical animal model: Galleria mellonella.

Materials and Methods. LAB's in vitro inhibition ability against Pseudomonas aeruginosa ATCC 27853 compared with a conventional antibiotic commonly prescribed in managing burn infections has been evaluated. Subsequently, the survival rate and the mRNA expression levels of antimicrobial peptides were monitored.

Results. LAB isolated from human breast milk demonstrated in vitro inhibition of P. aeruginosa. The G. mellonella burn infection model treatment improved the larvae survival rate and modulated the antimicrobial peptide expression in a strain-dependent manner.

Discussion and Conclusions. The in vitro antimicrobial activity of Lactic acid bacteria isolated from human milk was corroborated by the G. mellonella burn wound model, showing a protective and immunomodulatory in vivo. To sum up, this study opens promising avenues for further exploration and clinical translation of LAB implicated in burn wound care and for validating G. mellonella larvae as a potential burn wound model.

285 - SMALL MOLECULES AS MULTI-TARGET ANTIVIRAL AGENTS EFFECTIVE AGAINST A WIDE VARIETY OF VIRAL INFECTIONS

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Small molecules as multi-target antiviral agents effective against a wide variety of viral infections

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Introduction: Several strategies are currently available to develop small antiviral molecules for specific pathogenic viruses, including screening of compound libraries and drug discovery based on novel viral and/or cellular targets. New antiviral drugs based on innovative targets have strong competitive advantages, especially if they act as multi-target drugs. Here, we focused on the potential antiviral properties of three small molecule analogues, named RC368, RC370, and RC392, against herpes simplex 1 virus (HSV-1, neurotropic DNA virus), Influenza A virus (IAV, respiratory virus with a highly variable negative-sense RNA genome) and human coronavirus 299E (HCoV229E, respiratory positive-sense RNA virus).

Materials and Methods: Vero, A549 and Beas cells were infected with HSV-1, IAV and HCoV229E, respectively, and treated with the small molecules at different times of infection: before, during or after (post-infection) virus adsorption to the host cells. The efficacy of the infection was assessed by standard plaque assays, In-Cell Western assays, and RT-PCR. Cell extracts were analysed by western blotting. The virucidal activity was verified by pre-incubating the viruses with each compound and was confirmed by transmission electron microscope (TEM) analysis. The MTT assay was performed to test cell viability. The virological tests were performed with 20 µg/ml RC392 or 30 µg/ml RC368 and RC370.

Results: Our results showed that the post-infection treatment with RC368, RC370 and RC392 was highly effective in reducing the efficacy of HSV-1 infection by 1.5, 2, 1 log, respectively, as these compounds interfere with immediate early and early viral protein production. The great antiviral efficacy of RC368, RC370, and RC392 (70% reduction in virus titre) was also observed during the early stages of HCoV229E replication, suggesting that the small molecules are efficiently active during the synthesis of HSV-1 and HCoV229E viral functional proteins. In contrast, RC370 and RC392 showed no inhibitory effect on IAV infection, but RC368 treatment of cell monolayers during the pre- or post-absorption phases of IAV was effective in inhibiting its life-cycle (more than 50% reduction in virus titre). These results indicate that RC368 may either interact with the cell receptors exploited for virus entry in host cells, and affect the viral replication pathways. In addition, the small molecules showed an efficient virucidal activity, as confirmed by TEM analysis: RC370 and RC392 disrupted HSV-1 and HCoV229E envelopes, RC368 and RC370 destroyed IAV envelope and RC368 was also virucidal against HCoV229E virions.

Conclusions: Overall, our results indicate that RC368, RC70 and RC392 are multi-target antiviral agents effective against a wide variety of viral infections.

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Y

Z