SIM
SOCIETÀ ITALIANA
DI MICROBIOLOGIA

50° CONGRESSO NAZIONALE DELLA SOCIETÀ ITALIANA DI MICROBIOLOGIA

NAPOLI, 18-21 SETTEMBRE 2022 | HOTEL ROYAL CONTINENTAL

Abstract Book

CON IL CONTRIBUTO NON CONDIZIONANTE DI

SHIONOGI

Sommario

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5 - Formyl peptide receptors 1 and 2 are essential vita-PAMP sensors of bacterial pathogens

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Introduction: Host defense systems are differentially activated by the presence of live versus dead organisms during infection. *Therefore, previous studies have postulated* the existence of a class of Pathogen-Associated Molecular Patterns (PAMPs), called *vita*-PAMPs, that are specifically associated with viable bacteria and are capable of triggering distinctive responses in macrophages and dendritic cells. However, the molecular mechanisms underlying live-dead discrimination in neutrophils, the primary mediators of antibacterial host defenses, are still unknown.

Materials and Methods: We examined the role of Formyl peptide receptor 1 (Fpr1) and 2 (Fpr2), which sense the presence ofbacterial *N*-formyl peptides, using mice lacking these receptors in both *in vitro* and *in vivo* models of Group B Streptococcus (GBS) infection.

Results: *In vivo*, Fpr1- and Fpr2-defective mice were more susceptible to GBS infection relative to wild type mice, concomitantly with decreased production of the chemokine (CXC motif) ligand 2 (Cxcl2), impaired neutrophil influx and increased bacterial burden to infection sites. This phenotype was rescued by the exogenous administration of recombinant Cxcl2, but not Cxcl1, indicating that Fpr1/2 signaling is crucially required for robust Cxcl2 responses. *In vitro*, high-level production of Cxcl2 and reactive oxygen species (ROS) in neutrophils was elicited only by live, but not dead bacteria, and required both Fpr1 and Fpr2. Moreover, by systematically testing signal peptide sequences present in databases, we identified two distinctive sets of GBS signal peptides that could recapitulate the induction of high-level Cxcl2 responses when used in combination with toll-like receptor agonists.

Discussion and Conclusions: The simultaneous presence of agonists for Fpr1, Fpr2, and TLRs represents a unique signature associated with viable bacteria, which is sensed by neutrophils and induces high-level Cxcl2 production, Cxcl2-dependent autocrine cell activation and robust bactericidal activity. These results may be relevant to devise new alternative therapeutic strategies aimed at stimulating host defenses against antibiotic-resistant pathogens.

7 - PAD-mediated citrullination provides a new biomarker and an effective target to counteract
human papillomavirus transformation

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PAD-mediated citrullination provides a new biomarker and an effective target to counteract human papillomavirus transformation

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INTRODUCTION: Citrullination is an emerging post-translational modification catalyzed by peptidyl arginine deiminases (PADs) that convert peptidylarginine into peptidylcitrulline. In humans, the PAD family is composed of five isozymes (PADs 1-4, 6), ubiquitously expressed, and relevant to human diseases, including cancer.
MATERIALS AND METHODS: To characterize protein citrullination in the context of human papillomavirus (HPV) transformation, we took advantage of different *in-vitro* models of persistent high-risk HPV transformation *i.e.*, CaSki and HeLa cells, harboring integrated HPV-16 and HPV-18 DNA, respectively.
RESULTS: We demonstrated that the expression of E6 and E7 HPV oncoproteins is strongly impaired in the presence of the pan-PAD inhibitor BB-Cl-Amidine. Consistently, p53 and p21, the main targets of HPV oncoproteins, are upregulated by the PAD inhibitor, indicating that citrullination can be targeted to subvert HPV transformation. The overall citrullination nor the PADs profile are strongly affected by E6 and E7 expression in-vitro, but in-vivo analyses revealed a significant association between the presence of citrullinated proteins and cervical cancer progression. Interestingly, a higher PAD4, but not PAD2, score has been obtained in high-grade lesions, indicating that PADs could represent new suitable biomarkers for the disease.
DISCUSSION AND CONCLUSIONS: These findings could provide new insights into novel pathways beyond HPV-mediated cervical cancer progression.

13 - Effect of SARS-CoV-2 on the coagulation cascade in COVID-19 associated coagulopathies

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Effect of SARS-CoV-2 on the coagulation cascade in COVID-19 associated coagulopathies

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Background: Coagulation decompensation is one of the most frequent complications encountered in COVID-19 patients. In particular, the onset of thrombi and ischemia are often associated with a poor prognosis. Although the evidence for an association between SARS-CoV2 infection and coagulopathies has been already reported (Levi et al, 2020), to date the mechanism underlying the alteration of the coagulation cascade in some COVID-19 patients remains misunderstood. Recently, protein S (PROS1), an anticoagulant plasma protein involved in the correct homeostasis of the coagulation cascade, has been described as a potential risk factor for complications related to COVID19 (Lemke et al, 2020) and represents a potential target for PLpro SARS-CoV-2 enzyme proteolysis (Ruzika, 2020).

Aim: This study aims to investigate expression patterns in COVID19-associated coagulopathies, to identify possible pharmacological targets, focusing on PROS1 protein alteration.

Methods: Thrombotic, arteriosclerotic plaques, venous \ arterial, perivascular fat samples and blood samples were collected from COVID-positive and COVID-negative subject, and from COVID-positive subjects with no coagulopathies. SARS-CoV-2 presence will be evaluated by Real time PCR and by IHC and gene expression, and ELISA analysis will be performed to identify specific expression profiles associated with coagulation imbalances, with particular attention to protein S (PROS1).

Results and Conclusions: We found substantial differences in the activation of the coagulative cascade, and particularly a significant decrease of PROS1, in the COVID-19 cohort experiencing coagulative disorders, in association with SARS-CoV-2 positivity by IHC and real time PCR. These data suggested that, possibly, SARS-CoV-2 associated thrombotic/ischemic events might involve PROS-1 cleavage by viral PLpro, leading to the loss of its anticoagulant function. Basing on this evidence, the use of PLpro inhibitors might be suggest as a therapeutical tool for COVID-19 coagulopathies.

15 - Vaginal immunization by the human microbiota bacterium Streptococcus gordonii expressing a Chlamydia trachomatis antigen efficiently primes the immune system in mice

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Vaginal immunization by the human microbiota bacterium *Streptococcus gordonii* expressing a *Chlamydia trachomatis* antigen efficiently primes the immune system in mice

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Introduction: Mucosal surfaces are active immunological interfaces, and as such are promising avenues for vaccines delivery. However, their unique characteristics and the nature of immune system activation in these tissues require the utilization of appropriate adjuvants or delivery systems. Recombinant *Streptococcus gordonii* expressing a heterologous antigen can activate the immune system when delivered mucosally in mice, owing to its colonization capabilities. **Materials and methods:** In the present study, we examined whether intravaginal administration of a recombinant *S. gordonii* vector, named FR368, genetically engineered to express the *Chlamydia trachomatis* multivalent MOMP antigen, CTH522, could prime the immune system in a heterologous prime-boost scheme. Antibodies were quantified using ELISA. Presence and number of Antigen-specific Antibody Secreting Cells was quantified using B-cell ELISPOT. Plasma cells were quantified using Flow cytometry, staining for CD138 and TACI. Cytokines were assessed using *in vitro* stimulation of splenocytes, and intracellular cytokines staining using flow cytometry. **Results:** We show that the vaginal colonization itself triggered a systemic antigen-specific IgG response. Heterologous subcutaneous boosting with unadjuvanted CTH522 antigen at either three or six months after vaginal immunization resulted in a marked increase in serum CTH522-specific IgG levels in FR368-immunized mice, compared to WT-immunized ones. Concomitantly, a significant increase in the levels of CTH522-specific antibody secreting cells, alongside a rise in the percentage of plasma cells in the spleen and lymph nodes, respectively, were observed in the FR368-immunized mice. Vaginal colonization with FR368 also induced a shift in cytokines expression profile in antigen-specific T cells upon restimulation, dominated by an IL-17 increased expression, suggesting a mucosal association of antigen first encounter context. **Discussion and conclusions:** Our results highlight the potential of immunization with recombinant *S. gordonii* as a potent mucosal delivery system able to stimulate a systemic immune response in a heterologous prime-boost scheme, which is currently the focus of many vaccines' development.

30 - The two-component SaeRS system controls the interactions of group B streptococci with host cells by modulating PbsP expression

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The two-component SaeRS system controls the interactions of group B streptococci with host cells by modulating PbsP expression

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Introduction: *Streptococcus agalactiae*, also named Group B *Streptococcus* (GBS), is a commensal Gram-positive bacterium that colonizes the human gastro-intestinal tract and causes invasive infections in neonates, in the elderly and in patients with underlying chronic disease. Two-component signal transduction systems (TCSs), consisting of a sensor (S) histidine kinase and a response regulator (R), enable bacteria to adapt to a wide range of microenvironments. PbsP (Plasminogen binding surface Protein) is an important, highly conserved virulence factor of GBS that is markedly upregulated *in vivo*. This work was undertaken to study the functional role of SaeRS in PbsP regulation and virulence.

Materials and Methods: We introduced a single amino acid mutation in the *saeS* gene (T133A) and a full deletion of the *saeR* gene (Δ*saeR*) to obtain, respectively, constitutionally activated (phosphomimetic) and constitutionally de-activated systems in the background of the representative CC23 serotype III NEM316 strain. In these mutant strains, we have additionally deleted the *pbsP* gene to evaluate its role in these contexts. *In vivo* and *in vitro* gene expression was assessed in the mutants using RT-qPCR.

Results: The SaeS T133A mutant displayed a 100-1000 overexpression of *pbsP*, both at mRNA and protein levels, when grown under laboratory conditions compared to the parental strain. This mutant also displayed markedly increased adhesion to and invasion of human epithelial and endothelial cells, while parental adhesion levels were restored by *pbsP* deletion in the T133A background. Deletion of *saeR* markedly impaired the ability of GBS to persist *in vivo* and produce disease in mouse models of experimental infections, indicating a major role of the SaeRS system in virulence factor regulation.

Discussion and Conclusions: Collectively these data indicate that PbsP expression is markedly upregulated by SaeS activation, allowing GBS to adhere to an invade host barriers. Besides, *saeR* deletion results in complete loss of the ability of GBS to cause disease in different mouse infection models, indicating a major role of SaeR in virulence. These data suggest that the SaeRS TCS may represent a suitable target in alternative strategies to control GBS disease.

31 - Prevalence of hepatitis B virus infection among pregnant women: results from a hospital-based survey in Northern Italy.

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Prevalence of hepatitis B virus infection among pregnant women: results from a hospital-based survey in Northern Italy.

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Introduction. Although there is a safe and effective vaccine, hepatitis B virus (HBV) infection constitutes a global challenge, involving about one-third of the world's population with current or previous infection and possible progression to cirrhosis, liver failure, and hepatocellular carcinoma. Maternal-to-child transmission (MTCT) is the main HBV transmission route in many parts of the world, with a high risk to progression to chronicity. Screening programs aimed at identifying HBV surface antigen (HBsAg) positive mothers are part of routine examinations in most countries. In this study, the prevalence of HBV infection among pregnant women (PW) was evaluated in Parma (Northern Italy) from January 2021 to May 2022.
Materials and Methods. A total of 1,464 PW (771/1,464, 52.7%, Italian; 693/1,464, 47.3%, foreigners; 592/1,464, 40.4% inpatients; 872/1,464, 59.6%, outpatients) were examined. The median age of PW was 31.5 years ± 4.2 for Italian and 30 years ± 4.9 for foreigners. Laboratory diagnosis was carried out upon medical order. The diagnostic algorithm was aimed at assessing first anti-HBsAg antibodies (HBsAbs) (except in full-term or high-risk pregnancy, where only HBsAg was determined), and then HBsAg in case of a negative result for HBsAbs. Additional HBV serological markers were examined in case of HBsAg positivity. ARCHITECT chemiluminescent microparticle immunoassays (Abbott) were carried out, according to the manufacturer's instructions.
Results. Positive HBsAbs PW were 280 (280/1,464, 19.1%); 79.3% (222/280) were Italian and 20.7% (58/280) foreigners (79.3% *vs* 20.7% $P<0.0001$). Vaccinated Italian PW were 28.8% (222/771), while foreigners 8.4% (58/693) (28.8% *vs* 8.4%, $P<0.0001$). Unvaccinated and full-term/high-risk PW with negative HBsAg were 1,165 (1,165/1,464, 79.6%). PW positive for HBsAg were 19 (19/1,464, 1.3%); 18 were foreigners (18/19, 94.7%; median age 30.2 years ± 5.4) and one Italian (1/19, 5.3%, aged 37 years) (94.7% *vs* 5.3%, $P<0.0001$). PW positive for HBsAg had chronic infection with a negative result for HBeAg.
Discussion and Conclusions. This study found both a low prevalence (1.3%) of HBV infection among PW and a large part of the study population lacking protective HBsAbs. PW either unvaccinated or not responding to HBV vaccine imply periodical serological monitoring, with major impact on the national health system. Among HBsAg positive PW, foreigners prevailed (94.7%). Also considering the increasing migratory flows from HBV high endemic countries, continuous HBV monitoring and intensive vaccination campaigns are needed in order to reduce the risk of MTCT.

34 - Classification of Clostridioides difficile strains using a MALDI-TOF MS typing method.

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Classification of *Clostridioides difficile* strains using a MALDI-TOF MS typing method.

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Introduction. The epidemiology of *Clostridioides difficile* infection (CDI) changed over the last two decades since the emergence of *C. difficile* virulent strains, such as BI/NAP1/027 and BK/NAP7/078. In order to enhance the global surveillance system of this phenomenon, molecular typing methods are needed for epidemiological monitoring of CDI and for the identification and tracking of new emergent hypervirulent strains. This study evaluated the possibility to differentiate and classify by MALDI-TOF MS the different *C. difficile* strains circulating in our area using a statistical classifying algorithm model (CAM), in comparison with the PCR-ribotyping method. In addition, the MALDI-TOF MS approach was applied for typing of *C. difficile* strains isolated from an outbreak.
Materials and Methods. A total of 122 strains were used: 95 strains were submitted to PCR-ribotyping and used to create and validate the CAM and 27 strains, involved in a suspected outbreak and isolated after its creation, used to evaluate the performance of such CAM. For each strain, 400 µL of a 2.5 McFarland bacterial suspension were treated by heat shock and 15 µL of *C. difficile* DNA were added to a 35-µL reaction mixture for PCR-ribotyping by using two specific primers; 300 µL of the same suspension were subjected to protein extraction for MALDI-TOF MS typing (T-MALDI).
Results. The 95 *C. difficile* strains used during the first step were grouped in 10 ribotypes (PR1–PR10) by PCR-ribotyping. In particular, 93.7% of the isolates (89/95) were grouped in 5 ribotypes (PR1–PR5). For T-MALDI, two CAMs were tested: the first involved all 10 ribotypes whereas the second one only the PR1–PR5 ribotypes. A better performance was obtained using the second CAM: recognition capability of 100%, cross-validation of 96.6% and agreement of 98.4% with PCR-ribotyping results. During the second step, the 27 *C. difficile* isolates were grouped by T-MALDI in 3 different clusters, accounting for 21, 4 and 2 strains, respectively. The previously created CAM classified the 4-strains cluster as Ribotype 126, according to PCR-Ribotyping results. For the remaining 23 strains, the classification obtained by CAM was discordant with that obtained by PCR-Ribotyping, which identified ribotypes not included in the CAM.
Discussion and Conclusions. A validated CAM represents a useful epidemiological tool for the classification of the most frequently circulating ribotypes and for the clusterization of epidemiologically-linked *C. difficile* strains involved in outbreaks, even if related to ribotypes not included in CAM. A correct performance of the CAM requires its continuous updating on the basis of epidemiological data, in order to include new unknown circulating ribotypes.

37 - "Bacterial Outer Membrane Vesicles: from biology to vaccines and cancer immunity".

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"Bacterial Outer Membrane Vesicles: from biology to vaccines and cancer immunity".

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Abstract

OMVs are closed spheroid particles of 20-300 nm in diameter, released from all Gram-negative bacteria generated through a “budding out” of the outer membrane. In addition of having a multitude of fascinating biological functions, OMVs are emerging as a promising vaccine platform for three main reasons: (i) are readily phagocytosed by antigen-presenting cells which present OMV-derived peptides on MHC class II and MHC class I; (ii) carry many Microbe-Associated-Molecular Patterns (MAMPs), which stimulate innate and adaptive immunity; (iii) can be engineered with foreign antigens and can be easily and rapidly purified from culture supernatant.

Over the last few years our laboratory has been involved in the development of OMV-based vaccines against infectious diseases and cancer. First, we have applied novel strategies to engineer OMVs with heterologous antigens. Second, by using Synthetic Biology, we have created novel *E. coli* strains which release OMVs deprived of a large number of endogenous proteins. Such strains feature interesting properties which make them particularly attractive for vaccine design. Taking advantage of the availability such OMV-platform, a multi-component vaccine against *Staphylococcus aureus* is now ready to move to development. In addition, an anti-COVID-19 vaccine candidate which elicits robust protection against the original Coronavirus strain as well as the new emerging variants has been developed.

More recently, we accumulated sufficient experimental evidences supporting the notion that OMVs can play an important role in cancer immunity. In studying the mechanisms through which intestinal microbiome affects cancer development and immunotherapy, we demonstrated that the presence of microbial species expressing proteins with amino acid sequences homologous to cancer neoepitopes can inhibit tumor development in experimental mouse models. Such anti-tumor activity appears to correlate with the presence of neoepitope-specific T cells which accumulate in the lamina propria and in the tumor microenvironment. The existence of T cells with identical TCRs at both sites suggests that such T cells originate at the mucosal level and subsequently reach the tumor. Interestingly enough, if homologous epitopes are expressed in Gram-negative intestinal species, the anti-tumor activity can be at least partially mediated by the release of OMVs carrying such homologous sequences. We demonstrated that by artificially administered neoepitope-decorated OMVs to mice, epitope-specific T cells are elicited and animals are protected from tumor challenge. These data provide evidence of a broader OMVs biological function, which extends to cancer immunity via molecular mimicry. Such immunological role of OMVs would underline how inseparable evolution has made mammals and their microbiota; humans may be viewed as a single unit of evolutionary selection comprised of a host and its associated microbes. From a translation viewpoint, our data leads to the attractive hypothesis that OMVs engineered with cancer neo-epitopes could be exploited, in combination with other therapies such as checkpoint inhibitors, to potentiate the elicitation of cancer-specific T cell responses.

43 - Insight into the apoptosis mediated by feline herpesvirus (FeHV-1) on permissive cells

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Insight into the apoptosis mediated by feline herpesvirus (FeHV-1) on permissive cells

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1.Introduction:
Apoptosis, also known as programmed cell death, is a critical component of host defense mechanisms against viral infection, limiting viral replication and transmission. Multiple signaling pathways, both intrinsic and extrinsic, form a complex network to modulate apoptosis, even though viruses have distinct mechanisms for mediating or inhibiting apoptosis to avoid this process. In comparison to other herpesvirus members, little is known about the relationship between FeHV-1, the etiological agent of feline rhinotracheitis, and apoptosis. This study aimed to learn more about this connection by evaluating the impact of apoptosis on FeHV-1 infection of permissive cells.
2.Materials and methods:
Confluent monolayers of Crandell-Rees Feline Kidney Cell (CRFK) were infected using a MOI 1 at different time points. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was used to determine cell viability. Moreover, the apoptotic pathway was investigated using two different approaches: a flow cytometry analysis using an Annexin V-FITC detection kit and a western blot assay targeting specific markers of apoptosis (Caspase 8, Caspase 9, Caspase 3, Bcl-XL, Bcl-2).
3.Results:
Our results suggested that FeHV-1 triggers apoptosis process starting with 12 hours post infection. A larger percentage of early apoptotic cells were detected by the flow cytometry analysis, starting at 12 hours after infection, while late apoptotic cells increased at 24 hours after infection. The western blot analysis showed the cleavage of caspase 9 after 24 hours and a time-dependent cleavage of caspase 3 after 48 and 72 hours. We also observed a reduction of the expression of the anti-apoptotic proteins Bcl-XL and Bcl-2, beginning from 24 hours, also in this case.
4.Discussion and conclusions:
In this work we investigated on apoptosis modulation made by FeHV-1. Similar to other herpesviruses, such as Herpes simplex virus (HSV-1), Bovine herpesvirus (BoHV-1), Pseudorabies virus (PRV), Anatid alphaherpesvirus-1 (DEV), and Caprine Herpesvirus 1, infection on permissive cells results in the activation of the apoptotic process, which reduces cell viability. The induction of the intrinsic pathway and the caspase 9 cleavage suggested a dysfunction of the mitochondrial membrane, and the downregulation of Bcl-2 and Bcl-XL expression supported this hypothesis.
Even though additional research is needed to fully understand the viral mechanism and the viral genes/proteins involved in this process, this study provides new knowledge on the host-FeHV-1 interaction. The potential proviral effect may be examined since it has been shown that some viruses manipulate the cellular apoptotic mechanism to enhance their own dissemination within apoptotic bodies.

46 - Time-restricted feeding induces Lactobacillus- and Akkermansia-specific functional changes in the rat fecal microbiota

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Time-restricted feeding induces *Lactobacillus*- and *Akkermansia*-specific functional changes in the rat fecal microbiota

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1. Introduction

Diet is known to strongly influence composition and functions of the gut microbiota (GM), with key effects on host health. Time-restricted (TR) feeding is receiving growing attention, having been associated to numerous health benefits. The impact of TR feeding on the GM structure has been quite largely explored by metagenomics, but little is known about how this dietary regimen modulates GM protein functions. The aim of this study was thus to analyze the effects of TR feeding on GM functionality in a rat model, by measuring protein expression changes through a metaproteomic approach.

2. Materials and methods

Two groups of rats (8 rats each) were fed ad libitum or received TR feeding, respectively. After 48 weeks, stool samples were collected and divided into two fractions, then subjected, respectively, to DNA extraction, 16S rRNA gene amplification and DNA sequencing, and to protein extraction, filter-aided sample preparation (FASP) and liquid chromatography-high-resolution mass spectrometry analysis.

3. Results

The integrated metaproteogenomic approach used in this study allowed us to carry out both a taxonomic and a functional characterization of the GM. We observed that TR feeding has a relevant impact on numerous GM protein functions, involved in different biological processes and metabolic pathways. Specifically, the TR feeding regimen appeared to specifically boost *Akkermansia muciniphila* and to enhance proteolytic and galactose metabolism enzymes expressed by *Lactobacillus* spp.

4. Discussion and Conclusions

These data can help improve our understanding of relationships between diet, GM, and health, through a mechanism involving protein functions expressed by the potential probiotics *A. muciniphila* and *Lactobacillus*. Further studies integrating different levels of meta-omic investigations and functional analyses will be needed to further deepen our knowledge about molecular aspects of diet-induced changes in the human GM.

57 - Interferon beta augments the in vitro and in vivo microbicidal responses of neutrophils leading to clearance of staphylococcal infection.

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Interferon beta augments the *in vitro* and *in vivo* microbicidal responses of neutrophils leading to clearance of staphylococcal infection

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Introduction: We have previously found that gram positive and gram negative extracellular pathogens induce robust interferon-beta (IFN-beta) responses after sensing bacterial nucleic acids *via* endosomal toll-like receptors. The role of this cytokine and other type 1 IFNs in bacterial infection is complex and context dependent. We investigate here the role of endogenous and exogenous IFN-beta on the outcome of infection by *Staphylococcus aureus*, an antibiotic-resistant pathogen responsible for a high percentage of skin, soft-tissue and pulmonary infections.
Materials and Methods: We examined the role of endogenous IFN-beta by observing infection outcome in mice lacking IFN-beta in comparison with wild type animals, using a previously characterized dermatitis model. In addition, the effects of treatment with recombinant IFN-beta (rIFN-beta) were analyzed in *vivo* and in phagocyte cultures. Cytokine responses were evaluated by measuring protein concentrations by ELISA.
Results: The absence of IFN-beta significantly decreased host resistance to *S. aureus*, as evidenced -in skin lesions- by increased ulceration and bacterial numbers as well as decreased neutrophil infiltration and pro-inflammatory cytokine production. All these defects were rescued to wild type levels by local administration of rIFN. Moreover, rIFN increased resistance to infection also in wild type animals. The addition of the cytokine to bone marrow-derived macrophages and neutrophils and to whole blood increased in vitro killing of *S. aureus*. The effects on neutrophils were marked and were associated with an increased intracellular content and release of proteins with potent anti-bacterial activity.

Discussion and Conclusions: Our data reveal a protective role of endogenous and exogenous IFN-beta in the context of *S. aureus* skin infections. Notably, neutrophils were identified as the main target of the beneficial effects of this cytokine, which were linked here to increased production of anti-microbial proteins. Studies are underway to identify the neutrophil products predominantly responsible for IFN-beta-induced antibacterial activities. These data may be useful to devise alternative strategies to treat antibiotic-resistant infections.

59 - Consumption of traditional Sardinian fresh cheese casu axedu promotes changes in the rat gut microbiota composition and functions

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Consumption of traditional Sardinian fresh cheese *casu axedu* promotes changes in the rat gut microbiota composition and functions.

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1. Introduction

Fermented dairy foods are acknowledged for their health-promoting benefits, including obesity prevention and reduction of metabolic disorders risk, possible due to their production of bioactive metabolites and enrichment in beneficial microorganisms. Here, we aimed to investigate the effects of the consumption of *casu axedu*, a traditional Sardinian fresh cheese (FC), on the rat gut microbiota composition and functions.

2. Materials and Methods

We subjected rats to a diet supplemented with *casu axedu* (FC group, N=6) or unsupplemented (control group, N=6) for 8 weeks. Stools were collected at the beginning of the experiment and after 2, 4, and 6 weeks of treatment. Colonic luminal contents were also collected at the end of the experiment. 16S rRNA gene sequencing was performed on stool and colonic luminal samples to inspect the gut and FC microbiota structure. *Casu axedu* aliquots were also sequenced to evaluate their bacterial composition. Moreover, shotgun metaproteomics analysis was carried out on colonic luminal contents to evaluate the gut microbiota functionalities.

3. Results

FC supplementation led to an increase of several microorganisms of the intestinal microbiota after 8 weeks, including *Prevotella* and *Phascolarctobacterium*, as well as of specific FC members, such as *Lactococcus lactis* and *Leuconostoc mesenteroides*, already after 2 weeks of treatment. Metaproteomics led us to find many functions with a statistically significant difference in abundance between the two groups. In particular, the *Prevotella* proteome behaved differently based on the two different dietary treatments. Focusing on the microbial metabolic pathways, several differentially abundant enzymes were involved in the carbon metabolism pathway; of these, glycolysis- and pentose phosphate pathway-related enzymes were found as more abundant in the controls, whereas those involved in citrate cycle and propanoyl-CoA metabolism showed a higher abundance in the FC-fed rats.

4. Discussion and Conclusions

Our data illustrate the dynamics of intestinal microbiota taxonomic changes related to *casu axedu* consumption. Further, our metaproteogenomic approach provides evidence of the interplay between the bacteria and metabolites deriving from *casu axedu* assumption and the intestinal microbiota metabolism in the rat model.

61 - The immunological activity of Bacteroides thetaiotaomicron rough-type lipopolysaccharide

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The immunological activity of *Bacteroides thetaiotaomicron* rough-type lipopolysaccharide

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Introduction: *Bacteroides thetaiotaomicron* is one of the most extensively studied symbionts of the human gut and a long-time used model organism for functional microbiota research. Despite its widespread distribution among human populations, still very little is known about the role of bacterial cell envelope constituents in the crosstalk with the human immune system. Due to the extraordinary characteristic of this gut bacterium to produce multiple diverse capsular polysaccharides on its surface, most of research activities focused on defining how these complex polymers affect immune responses. This resulted in the drawback of neglecting another key cell surface glycoconjugate expressed by this Gram-negative, the lipopolysaccharide (LPS). LPS, in fact, is widely known to trigger a pro-inflammatory response in humans, whose potency is strongly dependent on the chemical structure of LPS itself. By taking advantage of an acapsular mutant of *B. thetaiotaomicron*, we evaluate the immunological activity of the rough-type LPS (R-LPS) produced by this gut mutualist using engineered human TLR4 and TLR2 reported cells model (HEK-Blue™ hTLR4; HEK-Blue™ hTLR2). **Materials and Methods:** HEK-Blue™ hTLR4 cells were seeded into 96-well plates (3×10^5 cells per well) and incubated with different concentrations of *B. thetaiotaomicron* R-LPS or *S. typhimurium* SH 2201 LPS (1, 10, 100 ng/mL) for 18 h to analyze Nuclear Factor kappa B (NF-κB) activation by evaluating NF-κB-dependent secreted alkaline phosphatase (SEAP) using QUANTI-Blue™. Likewise, HEK-Blue™ hTLR2 and HEK-Blue™ Null2™ cell lines were exposed to *B. thetaiotaomicron* R-LPS or *S. typhimurium* SH 2201 LPS (1, 10, 100 ng/mL) for 18 h, and for HEK-Blue™ hTLR2 cells also to Pam3CSK4 (500 ng/mL) and analyzed as above. **Results:** *B. thetaiotaomicron* R-LPS induced a significantly lower NF-κB activation compared to *S. typhimurium* LPS in HEK-Blue™ hTLR4 cells. Notably, the stimulation induced in HEK-Blue™ hTLR2 cells highlighted a potent dose-dependent activation by *B. thetaiotaomicron* R-LPS compared to

S. typhimurium LPS for which, as expected, no significant TLR2 activation was noticed. The lack of any NF-kB activation in HEK-Blue Null2 cells, indicated that NF-κB activation by *B. thetaiotaomicron* R-LPS were TLR-dependent. **Discussion and Conclusions:** The evaluation of the impact of this R-LPS on the human innate immune system *in vitro* revealed a weaker ability to engage the TLR4/MD-2/CD14 pathway compared to the LPS of the gut pathogen *Salmonella typhimurium*, while it was able to potently promote TLR2-mediated response. It would be interesting to evaluate the release of mediators after the interaction of this R-LPS with TLR2.

67 - The probiotic Lactobacillus rhamnosus LGG restrains the angiogenic potential of colorectal carcinoma cells by activating a pro-resolving program via Formyl Peptide Receptor 1

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The probiotic *Lactobacillus rhamnosus LGG* restrains the angiogenic potential of colorectal carcinoma cells by activating a pro-resolving program via Formyl Peptide Receptor 1

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Introduction: Chronic inflammation is a risk factor for Colorectal Carcinoma (CRC) onset, as strongly suggested by the increased predisposition to colon carcinogenesis of Inflammatory Bowel Disease patients. Intestinal inflammatory conditions are strongly influenced, and in turn affect, microbiota composition. Several studies in CRC patients and experimental models demonstrated that colon tumorigenesis is associated to significant alterations of intestinal microbial composition termed as dysbiosis. It is note that, *Lactobacillus rhamnosus GG* (*LGG*) is a commensal bacterium used as probiotic in order to reverse intestinal dysbiosis. Formyl Peptide Receptors (FPR1, 2, 3) are innate immune sensors of pathogen and commensal bacteria exerting a homeostatic role in colonic mucosa. FPR1, controls the production of pro-resolving mediators functioning as a tumor suppressor due to its ability to sustain an inflammation resolution response

with anti-angiogenic potential in gastric and in CRC cells. In this study, it was analysed the role of commensal *LGG* to promote intestinal epithelial homeostasis. It was investigated the possibility that *LGG*, interacting with FPR1, could activate a pro-resolving and an anti-angiogenic response in CRC cells. **Materials and Methods:** Colorectal carcinoma cells (HT29 and HCT116) were alternatively treated with bacterial supernatant (SN) of *LGG*, *Bifidobacterium bifidum*, a lactic acid bacteria and *Escherichia coli*, a non-probiotic bacteria, in order to evaluate the pro-resolving and anti-angiogenic effects. To verify this hypothesis, the levels of gene expression and production of pro-resolving and pro-angiogenic markers were evaluated by real-time PCR and ELISA assay. In addition, it was evaluated the ability of *LGG* SN to modulate CRC cell functional angiogenic potential through the *in vitro* Matrigel angiogenesis assays. To confirm that the pro-resolving and anti-angiogenic effects of *LGG* in CRC cells is FPR1-mediated, HCT116 cells stably expressing FPR1-short hairpin RNAs were treated with *LGG* SN. **Results:** Our results demonstrated that *LGG* can promote intestinal epithelial homeostasis through FPR1. A pro-resolving, anti-angiogenic and homeostatic functions were observed upon treatment of CRC cells with SN *LGG*, but not with SN of other lactic acid or non-probiotic bacteria. **Discussion and Conclusion:** The demonstrated beneficial effects of *LGG* are dependent on FPR1 expression, that could be a regulator of the balance between microbiota, inflammation and cancer in CRC model. These data also support the evidence that the pro-resolving and anti-angiogenic response in CRC cells is not general and common to all the commensal or to all the lactic acid bacteria.

68 - The Shigella flexneri virulence factor apyrase is released inside eukaryotic cells to manipulate host cell fate

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The *Shigella flexneri* virulence factor apyrase is released inside eukaryotic cells to manipulate host cell fate

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Introduction. *Shigella flexneri* is the causative agent of bacillary dysentery in humans. Its pathogenicity relies on the expression of type III secretion system that injects effector proteins inside host cell mediating bacterial internalization and cell response subversion. Apyrase is a periplasmic ATP-diphosphohydrolase required for *Shigella* actin-based motility whose catalytic role is still unknown. Caspase-mediated programmed cell death plays an essential role in the elimination of infected cells; it is tightly regulated by the intracellular levels of ATP. Here we hypothesized that apyrase could affect host cell survival by degrading the intracellular ATP. Materials and Methods. *S. flexneri* M90T, the *apy* mutant HND115, and the complemented HND115(pHND10) strains were grown in Luria-Bertani broth. Surface apyrase localization was analyzed by an immunodot-blot and western blot analyses. The human cell line Caco-2 was used for infection experiments, at a multiplicity of infection of 100, using the gentamicin protection assay; intracellular and extracellular ATP (iATP and eATP) quantification (ATP determination Kit Molecular Probes), caspase activity (Vybrant FAM poly Caspase Assay Kit), and cell death rates (eBioscience™ Annexin V Apoptosis Detection Kits) were measured. Results. The amounts of eATP during bacterial growth was significantly higher in the HND115 strain compared to those obtained from M90T and HND115(pHND10) strains, indicating that apyrase degrades eATP. Localization of apyrase on the outer bacterial surface was observed both in cultivated bacteria as well as in bacteria retrieved from infected cells at 4 hour post-infection (HPI). The iATP levels were significantly higher in Caco-2 cells infected with HND115 strain compared to those infected with M90T and the complemented strains at 3 HPI. Interestingly, cells infected with M90T and the complemented strains displayed ATP levels similar to the non-infected control cells. In addition, in these latter infected cells, apyrase was found to accumulate in the cytoplasm. This result indicates that apyrase is necessary to maintain the physiological levels of iATP during *S. flexneri* infection. Moreover, cells infected with HND115 strain showed a prominent increase in caspase activation compared to those infected with M90T and the complemented strains, as well as the non-infected control cells. Finally, the significant rise in the

percentage of Annexin V positivity by single and double staining with propidium iodide in HND115-infected cells suggests a possible activation of pyroptosis-mediated cell death. Conclusions. Our results indicate that apyrase reduces iATP content to keep caspase activation to basal levels, thereby promoting cell survival.

71 - Exploring the Nrf2-mediated antioxidant pathway and the potential use of redox-modulating compounds during respiratory virus infections

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Exploring the Nrf2-mediated antioxidant pathway and the potential use of redox-modulating compounds during respiratory virus infections

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Introduction: Changes in redox state homeostasis occur during respiratory virus infections and they can affect different steps of viral replication. The nuclear factor erythroid 2-related factor 2 (Nrf2)-pathway, involved in antioxidant genes transcription, is decreased during influenza virus infection and more recently, it was found downregulated upon SARS-CoV-2 infection. However, the mechanisms at the basis of this regulation are still not defined. At the same time, oxidative stress can also regulate the virus/host binding favoring viral entry. In this study we aimed at: i) exploring the mechanism and viral proteins involved in the regulation of Nrf2-mediated antioxidant response during influenza virus and SARS-CoV-2 infections; ii) evaluating the efficacy of redox-modulating compounds against both respiratory virus infections.

Materials and Methods: Permissive cell lines were infected with SARS-CoV-2, influenza A/PR/8/34 H1N1 virus (PR/8) or transfected with different PR/8 or SARS-CoV-2 plasmids encoding for selected respiratory virus proteins. The treatment was performed by using the n-butanoyl derivative of glutathione (GSH-C4) and I-152, a conjugate of N-acetylcysteine (NAC) or S-acetyl-cysteamine (SMEA), pre-incubating SARS-CoV-2 or PR/8 with the molecules for 1 h at 37° C or by adding the compounds after the viral adsorption for 24 h post-infection. Viral titration was performed by TCID50, hemagglutination and plaque assays. Viral proteins expression was analyzed by western blot and in cell western assay.

Results: The transfection with plasmids encoding for several influenza virus proteins, showed that the non-structural NS1 protein regulated the activation of antioxidant response element promoter and Nrf2 protein expression. A similar downregulation of Nrf2 pathway was found during SARS-CoV-2 infection, where ORF6 accessory protein modulated

the antioxidant pathway. Treatment with redox-modulating compounds added for 24 h post-infection, was able to rescue the Nrf2-mediated antioxidant response and to inhibit influenza virus replication. Interestingly, the same molecules inhibited the infectivity of SARS-CoV-2 by impairing both the binding of S protein to its cellular receptor during the early stage of viral infection, as well as the formation of new mature viral particles. The use of GSH precursors also interfered with PR/8 entry. Indeed, cells infected with a mixture of PR/8 and GSH-C4 showed a lower expression of viral proteins compared to untreated condition.

Discussion and Conclusions: These data contribute to clarify the mechanisms through which influenza virus and SARS-CoV-2 modulate the intracellular redox state to their advantage and to identify new possible cell-targeted strategies against respiratory virus infections.

80 - Drug-targeted activation of the RIG-I pathway to develop novel combinatorial therapeutic approaches against human papillomavirus-associated cancers

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Drug-targeted activation of the RIG-I pathway to develop novel combinatorial therapeutic approaches against human papillomavirus-associated cancers

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Currently, the treatment for (HPV)-associated cancers involves radiotherapy, chemotherapy or surgery, all with devastating effects on the targeted anatomical sites. Thus, alternative antiviral therapies with fewer side effects are urgently needed to improve patient outcomes. Restoring pathways capable of triggering immunogenic cell death (ICD) of HPV-associated tumors through production of type I interferons (IFNs) and lymphocyte-recruiting chemokines is of increasing interest. In this scenario, pattern recognition receptors (PRRs), which are proteins able to recognize conserved pathogen-associated molecular patterns (PAMPs) and initiate an antiviral response through NF-□B and interferon regulatory factor (IRF) signaling, appear to be among the most promising therapeutic targets. We have recently shown that HPV-transformed cells display marked downregulation of several PRRs, namely cGAS, RIG-I, and the downstream adaptor STING. Specifically, we found that HPV18 persistence in keratinocytes inhibits both type I and type III IFN production in response to DNA ligands, and that this effect is mainly due to suppression of the cGAS-STING pathway, which seems to be irreversible even after treatment with exogenous DNA. By contrast, the RIG-I pathway mediates the residual IFN production triggered by poly(dA:dT) or the sequence-optimized 5'pppRNA RIG-I agonist M8. In this regard, we have investigated the potential of the powerful RIG-I agonist M8 as an anti-cancer agent by analyzing its ability to induce cell death and activate the immune response in HPV16-transformed CaSki cells. We have demonstrated that M8 exerts an antiproliferative effect in CaSki cells that was enhanced upon combined treatment with the genotoxic agent cisplatin. This effect was almost abolished in CaSki cells stably knocked out for the RIG-I gene. In addition, conditioned media from M8-treated CaSki, but not CaSki RIG-I KO cells, significantly enhanced NK cell proliferation and CaSki cell killing *in vitro*. The same combined treatment was assessed in the preclinical murine syngeneic model of HPV-driven cancer based on dorsal subcutaneous (s.c.) injection of C3 cells, which harbor an integrated HPV16 genome, in C57BL/6J mice. Intratumoral M8 treatment significantly reduced tumor growth when compared to vehicle-treated tumors. This inhibitory effect was strongly enhanced when mice were co-treated intraperitoneally with cisplatin, when compared to

cisplatin or M8 alone. The immune infiltrate in the tumors treated with the combined therapy was significantly enriched in inflammatory monocytes and activated NK cells when compared to the single treatments. Our findings provide good evidence that drug-targeted activation of the RIG-I pathway is an attractive and feasible option to enhance the effectiveness of existing anticancer therapies.

87 - Pro inflammatory status and bacterial gut-vagina translocation in pregnant women affected by COVID-19

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Pro inflammatory status and bacterial gut-vagina translocation in pregnant women affected by COVID-19

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Introduction. Pregnant women are at increased risk for severe maternal and neonatal morbidity and mortality from viral diseases. The immune system during pregnancy is subject to changes that can lead to an increased risk of serious sequelae. This study aims to investigate the immunological and microbiological consequences of SARS-CoV-2 infection during pregnancy. **Materials and Methods.** During 2021-2022, at the Institute for Maternal and Child Health – IRCCS "Burlo Garofolo", Trieste, Italy, women diagnosed with SARS-CoV-2 infection during pregnancy were characterized for the presence of anti-SARS-CoV-2 IgG and the dosage of 27 soluble immune markers in sera and funicular blood samples at the time of delivery. In addition, the vaginal and rectal maternal microbiomes were profiled. At birth, newborns were tested for SARS-CoV-2 infection. Women were divided in three groups, "Early-CoV" negative for SARS-CoV-2 infection at time of delivery, "Late-CoV" positive during delivery, "Spread-CoV" positive during delivery and with other positive samples including vaginal, rectal, placental swabs and urine. **Results.** IgG dosage resulted more likely positive in funicular blood of the Early-CoV group (Chi-square p = 0.033), while no significant differences were observed in the maternal blood from different groups. In maternal blood, IL-8, IP-10 and MCP-1 increased in the Spread-CoV group compared with others. The G-CSF growth factor increased in maternal and funicular blood samples from the Spread-CoV group (p= 0.033). As regard the microbial profile, vaginal swabs from the Late-CoV and Spread-CoV groups showed higher alpha diversity. In addition, several bacteria, including *E. faecalis, Escherichia spp*. and *Prevotella spp*., were present both in vaginal and rectal swabs. Among newborns, a total of two positive nasopharyngeal swabs was identified in the Late-CoV and Spread-CoV groups. **Discussion and Conclusions.** The infrequent SARS-CoV-2 vertical transmission is likely due to the maternal antibodies transplacental passage. The vaginal dysbiosis observed at the time of delivery in Late-CoV and Spread-CoV groups could negatively affect newborns' health, while the shared bacteria between vaginal and rectal swabs suggested a gut-vagina translocation. To date, the emerging variants of the virus seem to be unrelated to the spread of SARS-CoV-2 to other tissues, though, this point needs further studies.

88 - The fetal environment can harbor bacterial DNA overlapping the microbiome of vaginal, rectal and oral maternal body sites

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The fetal environment can harbor bacterial DNA overlapping the microbiome of vaginal, rectal and oral maternal body sites

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Introduction. A controversy exists over the *in utero* microbial colonization hypothesis that challenges the clinical paradigm of the sterile womb. The debate is focused on the need to assure aseptic sampling and discriminate legitimate signals from contaminations in the sequencing outputs. **Materials and methods**. We profiled the microbiome of chorionic villi (CVS) and amniotic fluids (AF) and the matched vaginal, rectal, and saliva samples from 60 women undergoing villocentesis (n=23) or amniocentesis (n=37) for genetic reasons, maternal age or high risk screening tests. In addition, in CVS/AF samples and vaginal swabs, we dosed 27 immune soluble factors. **Results.** By filtering out the kitome (the reagents contaminations) observed in the 'no template controls' (n=24) from the taxonomic assignment of the biological samples, we identified 10/23 (43%) CVS samples positive for bacterial DNA and 12/37 (32%) AF positive samples. The ANCOM test identified bacteria significantly different between the negative and the positive samples, including *Anaerococcus* (W=551), *Corynebacterium* (W=560), *Dialister* (W=561), *Gemella* (W=554), *Haemophilus* (W=556), *Lactobacillus* (W=561), *Mobiluncus* (W=520), *Peptoniphilus* (W=552), *Porphyromonas* (W=561), *Prevotella* (W=561), *Streptococcus* (W=560), *Staphylococcus* (W=543), and *Veillonella* (W=558). These bacteria were identified in the positive CVS and AF samples, overlapping mostly the microbiome of the vaginal and rectal samples and, to a lesser extent, the saliva samples. In the vaginal swabs matching the CVS/AF positive samples, we identified a decreased concentration of the antiinflammatory FGF-beta growth factor (8±3 pg/mL) compared with the vaginal negative samples (13±15 pg/mL) (Mann-Whitney U test p=0.04). We did not identify significantly modulated immune factors in CVS/AF samples positive for bacterial DNA compared with the negative ones. **Discussion and conclusions.** Commensal and pathogenic bacteria can be identified in the placenta and amniotic fluid, deriving from several maternal body sites including the vagina, the oral cavity and the gut. The decrease of the antiinflammatory FGF-beta growth factor in the vaginal samples matching the colonized CVS/AF samples can facilitate the spread of these microbial constituents in the fetal environment. To identify potential new microbiome markers in the prenatal intrauterine environment can increase the knowledge of their putative role in human health.

90 - Specific microbiota compositions regulate humoral and cellular responses to vaccines in outbred mice

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Specific microbiota compositions regulate humoral and cellular responses to vaccines in outbred mice

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Introduction: Vaccination shows high variability in the elicited immune responses among individuals and populations, for reasons still poorly understood. An increasing number of studies are supporting the evidence that gut microbiota, along with other inter-playing variables, is able to modulate both humoral and cellular responses to infection and vaccination. With the aim of better understanding this connection, we have analyzed the antibody and cellular responses of outbred and inbred mice to several vaccines using different microbiota models.
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 mice with a panel of glycoconjugate or protein antigens two times at two weeks interval using alum as adjuvant. Antigen-specific antibody responses were monitored during the experiment by ELISA. Cellular immune responses were characterized by extracting draining and mesenteric lymph nodes, Peyer's patches and spleen to measure both frequencies of dendritic cells (DC), T and B cells populations and their immunophenotype by flow cytometry. To investigate the microbiota drivers related to the differential antibody response, we analyzed gut microbial-community structure by 16S rRNA amplicon sequencing.
Results: After vaccinating SW mice, antigen-specific IgG responses of SPF mice were significantly higher than GF or antibiotic-treated mice responses, for all antigens tested. In SW SPF mice, we observed an increased development of germinal center B cell and T cell activation as opposed to a higher regulatory/co-inhibitory environment in SW GF mice. By cohousing SW GF mice, we also found that the organisms responsible for an enhanced response to vaccination are both transmissible and ampicillin sensitive. Taxonomic analysis indicates that, at phyla level, high IgG responses in SW mice were associated with a marked increase of Firmicutes and a decrease in the Bacteroidetes. In agreement with the literature, we found less impact of the microbiota on the C57BL/6 mice vaccination response.
Conclusion: Overall, our data suggest that specific microbiota compositions can regulate humoral and cellular responses to vaccines in SW (outbred) mice, providing an important contribution to a better understanding of the microbiota-immune response crosstalk after vaccination.

91 - Persistence of spike-specific memory B cells and antibodies nine months after two doses of BNT162b2 mRNA vaccine

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Persistence of spike-specific memory B cells and antibodies nine months after two doses of BNT162b2 mRNA vaccine

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Abstract

Introduction. SARS-CoV-2 mRNA vaccines have demonstrated high efficacy and immunogenicity, but information on the durability of the humoral and memory B cell responses is needed. Here, we investigated RBD-specific memory B cells and humoral responses in healthy subjects, up to nine months after two doses of BNT162b2 vaccine (Comirnaty) administration.

Materials and Methods. Twenty healthy subjects, enrolled in the context of the IMMUNO_COV study (n. 18869, approved by local Ethical Committee), were follow up from day 0 up to 9 months after two doses of mRNA BNT162b2 vaccine (February-November 2021). Subjects infected with SARS-CoV-2 during this period were excluded from the present study. Spike-specific IgG were analysed by ELISA, while the ability of blocking the RBD-ACE2 binding was assessed using a surrogate of neutralization assay. RBD^+ B cells were identified by multiparametric flow cytometry, and data analyzed employing dimensionality reduction computational algorithm.

Results. Spike-specific antibodies were still present 9 months after two vaccine doses, even though a physiological reduction of titre was observed, with a stronger drop occurring in the first two months after the second dose. The frequency of subjects with antibodies inhibiting the RBD-ACE2 binding, decreased from 92.5% at month 3 to 80% at month 9. About 54% of subjects had IgG capable of recognizing the RBD of the Delta variant, while none of the subjects had antibodies capable of binding the RBD of Omicron variant. Three and nine months after vaccination RBD^+ B cells were still circulating, with a resting IgG^+ phenotype ($CD21^+CD27^+$), even though a small subset of IgA^+ was still detected. In most of the subjects, the in vitro restimulation, promoted the reactivation of B cells with differentiation into spike-specific antibody secreting cells.

Discussion and Conclusion. These data demonstrate vaccination with two doses of the RNA-based BNT162b2 vaccine elicits a strong B cell response with spike-specific memory resting B cells still persistent nine months after vaccination

95 - Depletion of the MS-ring protein FliF impacts motility, mucin-adhesion, and virulence in Bacillus cereus

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Depletion of the MS-ring protein FliF impacts motility, mucin-adhesion, and virulence in *Bacillus cereus*

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1. Introduction. In the last decades, the scientific scenario had pointed out the pivotal contribution of bacterial flagella to the virulence of some gastrointestinal pathogens. In previous studies, we showed that the deletion of genes encoding flagellar components and regulators in *Bacillus cereus* has a significant impact on motility, surface-adhesion, and secretion of virulence proteins. The aim of this study was to dissect the contribution of FliF, the unique constituent of the flagellar MS-ring, in *B. cereus* virulence.

2. Materials and Methods. In *silico* analysis of FliF in *B. cereus* ATCC 14579 (WT) was performed. RT-PCR experiments were conducted to verify the inclusion of *fliF* in an operon. A delta*fliF* mutant of *B. cereus* was constructed using an *in frame* markerless gene replacement method. Flagellation was evaluated by microscopy and SDS-PAGE of assembled flagellin. Swimming and swarming were analyzed in liquid media and on solid surfaces. The ability to form biofilms and to adhere to gastrointestinal mucins were verified by the crystal violet and mucin-adhesion assays, respectively. The secretion of hemolysin BL, proteases, and phosphatidylcholine-specific phospholipase C (PC-PLC) was tested by immunoblot and agar diffusion assays.

3. Results. *In silico* analysis of *B. cereus* FliF revealed that the protein shares conserved domains with FliF of other species that are required for its insertion in the plasma membrane and for the interaction with the C-ring. *fliF* was found to form a dicistronic operon with the upstream gene *fliE*. The delta*fliF* strain was found still able to assemble flagella, although in a reduced amount compared to the WT. However, motility was completely abolished by FliF depletion. While no differences in biofilm production were evidenced, FliF deprivation resulted in a reduced ability to adhere to mucins. Lastly, a significant reduction in the amount of secreted PC-PLC was detected in the supernatants of the delta*fliF* strain.

4. Discussion and Conclusions. Our results highlight a cardinal role of FliF in *B. cereus* virulence, emphasizing the linkage between flagella and pathogenicity in this organism. FliF is essential for motility and, therefore, for *B. cereus* ability to move toward suitable body sites and colonize host surfaces. The finding that FliF depletion reduces mucin-adhesion indicates an active role of flagella during gut colonization *in vivo*.

97 - Role of gut and oral microbiota in pediatric autism spectrum disorder

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Role of gut and oral microbiota in pediatric Autism Spectrum Disorder

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Introduction. Autism Spectrum Disorder (ASD) is a neurodevelopment disorder characterized by impairment in social interaction as well as sensory challenges. The gut microbiota in ASD patients revealed a high percentage of bacteria which seem negatively associated with the gut eubiosis. Recent studies on Alzheimer suggest that gut/oral bacteria metabolites (short chain fatty acids – SCAFs) may invade the blood-brain barrier (BBB) triggering inflammatory response, upregulation of microglia and alteration of metabolic activity in the CNS. This study aims to profile gut/oral bacteria of ASD children investigating the role of metabolites and cytokines linked to key bacteria in order to characterize possible relation with patient's metadata as well as the severity of the ASD clinical diagnosis. **Materials and Methods.** 35 matched stool and oral samples, collected after a teeth cleaning session, from pediatric children including 13 ASD and 24 no-ADS controls (average age: 7 years old) have been sequenced with PGM Ion Torrent. Eating habits have been additionally collected. Data have been analyzed through R dada2 pipeline and Blastn and for statistical analysis specifical scripts were set up using Python libraries. **Results.** The analysis of the ASD microbiota composition at genus level compared to no-ADS group showed a decreased level in *Bacteroides*, *Bifidobacterium* and *Dialister* while *Sutterella* and *Alistipes* genera incremented. At specie level, *S. wadsworthensis*, which has already been associated with CNS and GI diseases as well as inflammation, was at a higher level in ASD group, while *A. muciniphila* and *B. adolescentis*, which were associated to beneficial effects, decreased. The analysis of the oral ADS microbiota showed a lower level of *Porphyromonas*, *Fusobacterium* and *Leptotrichia*, while pathogen genera like *Haemophilus* and *Streptococcus* levels increased. **Discussion and Conclusions.** So far, these preliminary results showed a specific microbiome composition of the gut and the mouth of ASD children independently of diet, with higher levels of bacteria already linked with inflammatory conditions and described in adult neurological diseases. Further analyses are going to be performed focusing on the role of SCFAs on BBB permeability and the inflammation level of the CNS.

102 - Influenza virus-induced APE1 downregulation: implications for NRF2 pathway and host immune response

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Influenza virus-induced APE1 downregulation: implications for NRF2 pathway and host immune response

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Introduction

The influenza virus infection causes intracellular redox imbalance important for the activation of specific pathways involved in the control of viral replication. As an example, the nuclear factor erythroid 2-related factor 2 (NRF2) pathway regulates the antioxidant response; its expression is downregulated during the infection, although the mechanisms of this modulation are still unknown. One of the NRF2 interactor is the apurinic/apyrimidinic endonuclease1 (APE1); it has been reported that APE1 is required for the maintenance of NRF2 stability. Interestingly, APE1 redox function has been also involved in the activation of inflammatory factors in lipopolysaccharide-induced inflammation models. On the basis of this evidence, the aims of the study were: i) to evaluate the expression of APE1 during influenza virus infection and to correlate its expression with that of NRF2; ii) to evaluate the virus-elicited inflammatory response in APE1-inhibited or silenced conditions.

Methods

Human Bronchial Epithelial cells (BEAS-2B) were infected with influenza virus A/Puerto Rico 8/34 H1N1. Protein and mRNA expression was evaluated by western blot and q-RTPCR. Silencing of APE1 was performed by small interfering RNA (siRNA) assay. Pro-inflammatory cytokine levels were quantified by Luminex assay.

Results

NRF2 protein levels were downregulated by 55% after 24 h infection with respect to the mock-infected control, while the mRNA levels were downregulated by the 25%. At the same conditions, APE1 protein expression was reduced by 45% and mRNA levels by 21%. To evaluate an eventual relationship between both the proteins, in APE1-silenced infected cells (46% downregulation) NRF2 was further inhibited (42% downregulation), thus suggesting that the downregulation of APE1 could be a mechanism by which NRF2 is modulated during influenza virus infection.

To study the role of APE1 in the inflammatory response, cells were infected for 1 h and then treated or not with a molecular inhibitor of the APE1 redox activity, E3330. Levels of Interferons alpha and beta, RANTES and Interleukin-6 were induced after 24 h infection (2.7, 60, 349 and 1807 pg/mL, respectively), but their amounts were strongly decreased in the E3330-treated samples (1.6, 29, 167 and 1247 pg/mL, respectively). A similar modulation of cytokines and chemokines was found in APE1-silenced cells with respect to the scrambled controls.

Conclusions

The results indicate a downmodulation of APE1 during influenza virus infection and suggest its relationship with the drop of NRF2 expression. Even though further studies are needed to evaluate an eventual role of APE1 in controlling viral replication, data show that APE1/REF-1 redox function is capable of regulating the influenza virus-elicited inflammatory response.

103 - Results from a large cross-sectional Italian study assessing Ureaplasma spp. and Mycoplasma spp urogenital infections among STI clinic attendees and couples with primary infertility.

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Results from a large cross-sectional Italian study assessing Ureaplasma spp. and Mycoplasma spp urogenital infections among STI clinic attendees and couples with primary infertility.

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Introduction. Mycoplasmas are frequently isolated from the genital tract and the majority of individuals do not develop any disease. In STI (Sexual Transmitted Infections) patients, the routine testing and treatment of asymptomatic or symptomatic patients with *M. hominis*, or *U. urealyticum* or *U. parvum* is not recommended whereas detection of the other STI agents is suggested . Female and male infertility have been associated to *Ureaplasma spp*. and *M. hominis* urogenital infections. In Italy, evidence from large studies assessing their prevalence and putative associations with symptoms and demographic characteristics among STI and infertility patients belonging to the same geographic area is still scarce. **Material and Methods**. The study design was a retrospective cross-sectional study (from 2015 to 2021) including 4372 subjects (2647 female, m.a 36y and 1725 male, m.a. 35y), 2053 with a diagnosis of primary infertility (PMA-group) and 2319 STI clinic attendees, all tested for *M. hominis/genitalium*, *U. urealyticum/parvum*, *C.trachomatis*, *N.gonorrhoeae* and *T.vaginalis* by a multiplex molecular PCR. Quantitative bacterial vaginosis (BV) was performed in female subjects. Clinical and demographic data were collected for all subjects. **Results.** The overall prevalence of *Ureaplasma spp*. was higher in PMA-group. In this serie, *U. parvum* was detected at 23,77% *vs* 10,47% of STI ($p<0.001$) and *U. urealyticum* at 27% *vs* 19.5%. Conversely, *Mycoplasma spp*. was higher in STI patients. *M. hominis* was the most frequently detected (12% in STI *vs* 3% of the PMA-group) while *M. genitalium* was detected at 5.6% *vs* 0.35% respectively ($p<0.001$). Coinfection with other STI or BV was very low in both groups showing *C. trachomatis* the microorganism most frequently detected. In both series, the prevalence by sex showed that infections occurred more frequently in female (65%) and the age range of 25y-34y was the most affected (31%). Casual sexual intercourse was significantly identified as risk factor for *U. urealyticum*, *M. hominis* and *M. genitalium* ($p<0.001$) but not for *U. parvum*. Although the majority of subjects were asymptomatic (79%) the presence of symptoms not linked to other STI were significantly associated with *M. genitalium* in both sex. From 2020 to 2021 an increased trend of symptomatic STI patients has been observed for *M. hominis/U. parvum* in female ($p<0.001$) and for *U. Urealiticum* in male ($p<0.001$). **Discussion and Conclusion**.The overall data showed an increase of these microorganisms as single infections in both STI and PMA-group. These evidence indicate the need for further investigation on the role of these microorganisms in the different clinical setting.

114 - Human cytomegalovirus infection drives a cellular senescence-like phenotype in kidney epithelial cells

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Human cytomegalovirus infection drives a cellular senescence-like phenotype in kidney epithelial cells

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INTRODUCTION. Human cytomegalovirus (HCMV), a member of the Betaherpesvirinae subfamily, is one of the most frequent viral pathogens associated with acute kidney injury and kidney rejection. Although a small number of studies are available, mounting evidence suggests the activation of senescence responses upon viral infections. Cellular senescence is a state of stable cell cycle arrest associated with macromolecular alterations and secretion of pro-inflammatory cytokines and molecules. Senescence-associated phenotypes restrict damage propagation and activate immune responses, two essential processes involved in response to viral infections. Senescent cells accumulate in the kidney due to aging, any insult causing acute kidney injury, including post-transplantation, and chronic kidney disease. In each of these broad settings, higher levels of senescent cells associate with worsened kidney function and outcome. Our group previously reported that HCMV infection in human fibroblasts triggers cell senescence, and here we show primary kidney proximal tubular epithelial cells (RPTEC) undergo cellular senescence in order to support complete viral replication upon HCMV infection.
MATERIAL AND METHODS. RPTECs and the kidney adenocarcinoma 786-O cell line were infected with HCMV strain TR. Fate of infection was evaluated through time course morphological analysis, western blotting for viral protein expression and flow cytometry. A transcriptome analysis was performed in both cell lines at 2dpi upon infection, then the senescent profile was assessed in RPTECs and human foreskin fibroblast (HFF) *in vitro*. Using tissue sections derived from a full-blown HCMV infection in a preterm child, we show a large number of tubular epithelial cells in kidney expressing both HCMV proteins and the senescence marker lipofuscin.
RESULTS. We experimentally demonstrated that RPTECs, but not a renal cancer derived-cell line, fully supports HCMV replication and undergoes a senescence program upon infection that triggers a harmful secretory phenotype with the

ensuing induction of paracrine senescence in uninfected surrounding cells. Notably, the hallmarks of senescence-related inflammation found in both infected and uninfected RPTECs, that were not observed in HFFs, suggest that this virus-evoked senescence may contribute to disease pathogenesis at least when it comes to renal tubular cells that are a classical site of HCMV replication

DISCUSSION AND CONCLUSION. Altogether, we define novel pathogenetic mechanisms of renal injury upon HCMV infection that involve induction of a senescence program which in turn may pave the way for novel intervention strategy to counteract HCMV-related kidney disease.

115 - Three-label host-cell adhesion assay to assess the exclusion effect exerted by Lactobacillus acidophilus on adhesion of Pseudomonas aeruginosa to A549 alveolar epithelial cells

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Three-label host-cell adhesion assay to assess the exclusion effect exerted by *Lactobacillus acidophilus* on adhesion of *Pseudomonas aeruginosa* to A549 alveolar epithelial cells

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Introduction: There is increasing evidence that *Lactobacillus* strains can exert protective effects outside their traditional field of application i.e. the gut. As part of a project aimed at evaluating the aerogenous administration of probiotics for the treatment/prevention of pulmonary infections in cystic fibrosis (CF), in this study, we assessed the ability of different *Lactobacillus* strains to prevent adhesion of clinical isolates of *Pseudomonas aeruginosa* to the human alveolar A549 epithelial cell-line.

Materials and Methods: A panel of 8 commercial *Lactobacillus* strains was investigated for their adhesion capacity to A549 cells at a MOI of 10:1 *Lactobacillus* per cell. For exclusion experiments A549 were incubated with *Lactobacillus* spp for 2h, washed 3 times to remove non-adherent bacteria, and incubated with *P. aeruginosa* at a 10:1 MOI for 1h. The wells were washed twice and CFU counts were assessed. Image analysis was performed via the Operetta CLS High Content Analysis System following single or triple labeling of cells/bacteria with long tracking fluorescent dyes.

Results: *L. acidophilus* and *L. plantarum* displayed the highest adhesion ability evaluated as percent of adhered bacterial cells as compared to the inoculum. Dose-response experiments identified the MOI of approximately 100:1 as the best compromise between *Lactobacillus* adherence to and cytotoxicity towards A549 cells. In agreement with the observed adhesion capacity, *L. acidophilus* was the most efficient in preventing adhesion of a *P. aeruginosa* strain isolated from CF sputum, followed by *L. paracasei*, *L. rhamnosus*, *L. fermentum* and *L. plantarum*. Three-color fluorescence labeling of A549 cells, *P. aeruginosa*, and *L. acidophilus*, and confocal image analysis by Operetta system revealed a clear ability of *L. acidophilus* to prevent adhesion of *P. aeruginosa* to A549 cells, in exclusion experiments. Such results were confirmed by CFU count.

Discussion and conclusions: Diversity was observed in the adhesion properties of various *Lactobacillus* strains to human lung epithelial cells. Based upon such adhesive properties, *L. acidophilus* was identified as an interesting candidate that will undergo further studies as potential probiotic for aerogenous administration. The triple-fluorescence staining and the computer-assisted quantitative analysis via the Operetta system revealed a feasible and high sensitive method that can simultaneously probe and localize the relative adhesion of *Lactobacillus* and pathogens onto host cells. Such method may represent an important tool for studying the complex interplay between bacterial pathogens and host.

The study received support from the Italian Cystic fibrosis research foundation, Project FFC#13/2021.

118 - Recall immune responses after murine respiratory pneumococcal infection are detected by in vitro restimulation of splenocytes with Streptococcus pneumoniae inactivated whole cells

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Recall immune responses after murine respiratory pneumococcal infection are detected by *in vitro* restimulation of splenocytes with *Streptococcus pneumoniae* inactivated whole cells

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Introduction
The *in vitro* stimulation of immune system cells with live or killed bacteria is essential for understanding the host response to pathogens. In the present study, we propose a model combining transcriptomic and cytokine assays on murine splenocytes to describe the immune recall in the days following pneumococcal lung infection.
Materials and Methods
Mice were sacrificed at days 1, 2, 4, and 7 after *Streptococcus pneumoniae* (TIGR4 serotype 4) intranasal infection and splenocytes were cultured in the presence or absence of the same inactivated bacterial strain to access the transcriptomic and cytokine profiles.
Results
The stimulation of splenocytes from infected mice led to a higher number of differentially expressed genes than the infection or stimulation alone, resulting in the enrichment of 40 unique blood transcription modules, including many pathways related to adaptive immunity and cytokines. Together with transcriptomic data, cytokines levels suggested the presence of a recall immune response involving both innate and adaptive immunity, stronger from the fourth day after infection. Dimensionality reduction and feature selection identified key variables of this recall response and the genes associated with the increase in cytokine concentrations.
Discussion and Conclusions
This model could study the immune responses involved in pneumococcal infection and monitor vaccine immune response and experimental therapies efficacy in future studies. A set of possible biomarkers of early and late infection was identified.

119 - Antimicrobial peptide human beta-defensin-2 reduces the gut inflammation induced by enteroinvasive Escherichia coli by inhibiting invasion and virulence factors expression

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Antimicrobial peptide human beta-defensin-2 reduces the gut inflammation induced by enteroinvasive *Escherichia coli* by inhibiting invasion and virulence factors expression

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Introduction: the intestinal microbiota consist of a community of about 100 trillion of mutualistic microorganisms that contributes to homeostasis and to development of the intestine by regulating various metabolic functions of the host, such as digestion and the supply of nutrients. One of the species most represented in the intestinal microbiota is *Escherichia coli*, a Gram-negative bacillus that, as commensal, colonizes expecially the lower tract of the human intestine. However, some strains of *E. coli* can acquire, through the horizontal transfer of DNA, a virulence factors which making them able to adapt to new intestinal niches. Among these, enteroinvasive *E. coli* (*EIEC*) is responsible for the bacillary dysentery that causes diarrheal symptoms similar to shigellosis in both children and adults, particularly in low-income countries and in poor hygiene conditions. The indiscriminate use of antibiotics has led to the development of bacterial resistance which represents a danger to global public health. In this scenario, antimicrobial peptides (AMPs) have received widespread attention due to their broad antimicrobial spectrum and low incidence of resistance phenomena. Furthermore, AMPs modulate the immune defenses of the host and regulate the composition of microbiota and the renewal of the intestinal epithelium. Aim of this work was to create a line of intestinal epithelial cells able to express high concentrations of antimicrobial peptide human beta-defensin-2 (HBD-2), to test its ability to interfere with the pathogenicity mechanisms of *EIEC*. Materials and Methods: *Cell cultures*: gene coding HBD-2 was cloned and transfected into Caco-2 cells which were subcultured for 21 days to obtain their full differentiation. *Infection and Real-Time PCR:* the transfected and untransfected cells were infected with *EIEC* and the modulation of expression levels of pro- and anti-inflammatory cytokines, and of bacterial virulence factors was evaluated by Real-Time PCR. *Invasiveness assay:* transfected and untransfected cells were infected with 10^8 CFU/ml of *EIEC* for 2 hours at 37°C, then were treated with gentamicin at bactericidal concentration for additional 2 h, and finally lysed, serially diluted and incubated overnight at 37 °C to identify the viable intracellular bacteria. Results: HBD-2 is able to significantly reduce the expression of the proinflammatory cytokines and the invasiveness ability of *EIEC*. The molecular analysis on the bacterial genes associated with invasive ability shows a constant reduction in the expression of all virulence factors in presence of HBD-2.Discussion and Conclusions: These findings set the stage for the search for alternative natural therapeutic solutions for the treatment of difficult-to-treat infections.

123 - Epstein-Barr virus and Mycobacterium avium subsp. paratuberculosis homologues peptides elicit a strong humoral response in patients with neuroinflammatory disorders, and exacerbate EAE

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Epstein-Barr virus and _Mycobacterium avium_ subsp. _paratuberculosis_ homologues peptides elicit a strong humoral response in patients with neuroinflammatory disorders, and exacerbate EAE

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1. Introduction

Neuroinflammation can be induced by pathogens infection such as bacteria and virus, however, their pathological role still unclear. Here, we characterized antibodies against *Epstein-Barr* virus (EBV) nuclear antigen 1 (EBNA1), *Mycobacterium avium* subsp. *paratuberculosis* (MAP) heat shock protein (HSP) 70, and human central nervous system protein GlialCAM homologues peptides in blood samples of Japanese patients with different neurological disorders. Furthermore, we tested the effects of these peptides in active experimental autoimmune encephalomyelitis (EAE), a common animal model of neuroinflammatory disorders.

2. Material and Methods

Forty-seven patients with multiple sclerosis (MS), 32 with neuromyelitis optica spectrum disorder (NMOSD),19 with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), 32 disease controls, and 70 healthy controls, were retrospectively tested using indirect and inhibition ELISAs. Group of female C57BL6J mice received were immunized with the pathogen-derived peptides two weeks prior active EAE induction using myelin oligodendrocyte glycoprotein.

3. Results

A significantly strong antibody response against $EBNA1_{386-405}$, $MAPHSP70_{533-545}$ and $GlialCAM_{370-389}$ was detected in the serum of patients with MS and NMOSD in comparison to patient with MOGAD, and both control groups. A good direct correlation between antibody titers was evidenced in patients with MS and NMOSD. Moreover, competitive immunoassay demonstrated the presence of cross-reactive antibodies to EBV and MAP antigens. No significant correlation was found between antibody titers and clinical therapy, aquaporin-4 antibody titer.

Following immunization with $EBNA1_{386-405}$ and $MAPHSP70_{533-545}$ peptides, mice showed early onset and more severe disease in comparison to no treated mice.

4. Discussion and Conclusions

We demonstrated a strong humoral response and the significant presence of cross-reactive antibodies against EBV and MAP derived peptides in patients with MS and NMOSD, as well as the encephalitogenic potential of these peptides in EAE model, providing evidence for an association between these pathogens and different demyelinating disorders. Molecular mimicry could be the mechanism by which EBV and MAP can induce autoimmunity in genetically susceptible individuals.

129 - Salmonella Infantis releases Outer Membrane Vesicles (OMVs) including beta-lactamases enzymes during their biogenesis

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***Salmonella* Infantis releases Outer Membrane Vesicles (OMVs) including beta-lactamase enzymes during their biogenesis.**

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Introduction: Outer membrane vesicles (OMVs) are spherically bilayered nanoparticles, released into extracellular milieu by Gram-negative bacteria. OMVs originate from the outer membrane, contain different cargo molecules and mediate several biological processes. Recent studies have shown that OMVs are involved in antibiotic resistance (AR) mechanisms by including beta-lactamase enzymes, which degrade beta-lactam antibiotics, in their lumen. Recently, multidrug resistant and extended-spectrum beta-lactamases (ESBLs) *Salmonella* Infantis strains have spread widely in Europe. Since no studies have been conducted on *S.* Infantis' OMVs yet, the aim of the work was to collect OMVs from *S.* Infantis beta-lactam resistant and susceptible strains and to investigate whether beta lactamase enzymes are included in OMVs during their biogenesis by measuring the beta-lactamase enzymes activity in the OMVs. **Materials and Methods**: OMVs were isolated through filtration and ultrafiltration of culture supernatants. To investigate the presence of beta-lactamase enzymes into OMVs, beta-lactamase activity was quantified by Nitrocefin assay in three different samples: the filtered supernatants, the eluted samples from ultrafiltration, and then OMVs concentrates. Transmission electron microscopy (TEM) was used to investigate OMVs morphology. To normalize beta-lactamase activity value proteins from all samples were quantified by Bradford assay. Anti-beta-lactamase antibody was used to confirm the presence of beta-lactamase enzymes into OMVs. Statistical analysis was applied. **Results**: in the study we found that both beta-lactam and susceptible strains release OMVs. TEM images showed round shaped vesicles, from 60 to 230 nm, mainly organized in clusters with an electrodense appearance. No beta-lactamase activity value was detected in OMVs from susceptible strains. A very low value due to free beta-lactamase and OMVs was measured in filtered supernatants. The eluted samples showed a low activity value for the presence of free beta-lactamase. A significantly higher value than in the filtered and eluted samples, was obtained in OMVs concentrates. Anti-beta-lactamase antibody confirmed the presence of beta-lactamase enzymes in the lumen of the OMVs. **Discussion and Conclusions**: these results suggest that beta-lactamase enzymes also get packaged into OMVs from bacterial periplasm during OMVs biogenesis. A possible explanation could be that enzymes are loaded into OMVs to be protect from proteases degradative action. The possibility of investigating and understanding whether OMVs play a role in the mechanisms of AR opens the way towards the chance of developing new therapeutic strategies to fight antibiotic resistance in the future.

133 - Creation of an in vitro model for the screening of different probiotic strains able to prevent to inflammatory diseases of the gastrointestinal system

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Creation of an *in vitro* model for the screening of different probiotic strains able to prevent to inflammatory diseases of the gastrointestinal system

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Introduction**:** the integrity of intestinal barrier is fundamental for gut health and homeostasis; this condition is maintained by a mucus layer of epithelial cells held together by a complex system of intercellular junctions including weak and apical protein complexes that called "tight junctions" (TJs); TJs are composed of several multiprotein complexes including transmembrane proteins such as Claudin and Occludin, Junctional Adhesion Molecules and others, which interact with each other and with intracellular scaffold proteins, including Zonula Occludens, which are anchored to the actin cytoskeleton. These interactions control the passage of solutes, water, large molecules, and ions through the extracellular space. Alterations to TJs are associated with changes in epithelial permeability, which can lead toseveral diseases, such as inflammatory bowel disease, irritable bowel syndrome, celiac disease, diabetes, diarrhea, atopic eczema, and altered sensitivity to food allergens. *Lactobacillus* spp. are naturally present in the human intestinal tract, and several species and strains have been evaluated for their probiotic activity. The enhancement of epithelial barrier function is one of the proposed mechanisms by which certain probiotic organisms may confer beneficial activities. In this study we performed an intestinal co-cultures model, which provides a mucus-coated epithelial monolayer able toreproduce the condition that occurs *in vivo*, treated with different strain of *lactobacilli*, to establish the strains most active in strengthening the integrity of the barrier**.** Materials and methods**:** adenocarcinoma colorectal Caco-2 and HT29-MTX mucus-secreting epithelial cells were plated in 12-well Transwell® with polycarbonate insert, in a 7: 3 ratio Caco-2: HT29-MTX and cultured in DMEM medium with 10% fetal bovine serum, 100 IU / ml penicillin, 100mg / ml streptomycin and 2mM glutamine, at 37 ° C in a 5% CO_2 for 21 days to obtain their differentiation. Co-cultures were then treated with the 0.5 O.D. suspensions of *Lactobacillus brevis*, *Lactobacillus fermentum*, *Bifidobacterium lactis*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Lactobacillus paracasei*, cultivated at 37 ° C in microaerophilic conditions in MRS broth. The infection was carried out for 6 hours, then the expression of the tight junctions Zonulin-1, Claudin-1 and Occludin genes was evaluated by Real-Time PCR. Results: all strains tested-are able to significantly induce the expression of all TJs genes. Discussion and Conclusions: these data suggest the possibility to use specific formulations based on selected probiotic strains for the treatment and prevention of the reduced epithelial barrier function found in many disorders.

135 - Limosilactobacillus fermentum from buffalo milk inhibits Helicobacter pylori in a gastric epithelial cell model

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***Limosilactobacillus fermentum* from buffalo milk inhibits *Helicobacter pylori* in a gastric epithelial cell model.**

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Introduction: *Helicobacter pylori*, a Gram-negative microorganism, is recognized as a major cause of most gastroduodenal diseases, including gastric adenocarcinoma and gastric cancer. Prevailing therapy as the primary treatment of *H. pylori* infection involves a proton pump inhibitor and antibiotics (amoxicillin and clarithromycin), but this treatment can cause serious side effects. Therefore, probiotics, defined as "live microorganisms which, when administered in adequate quantities, can exert a health benefit to the host", have been studied as an adjuvant therapy modality. The aim of this study was to evaluate the role of a strain of *Limosilactobacillus fermentum* isolated from buffalo milk during *H. pylori* infection in gastric epithelial cells. Materials and methods: human gastric adenocarcinoma cell line AGS cells were cultured in Ham's F-12 K medium supplemented with 1% Penstrep, 1% glutamine and 10% fetal calf serum at 37°C and 5% CO_2. The isolated strain of *L. fermentum* was identified by molecular analysis with amplification of specific sequences. Before the infection, the strain was grown anaerobically in MRS broth at 37°C for 24-48 h, centrifuged at 4.000 x g for 10 min, washed twice with saline solution, resuspended at the concentration of 0.5 OD_{600} (10^8 CFU/ml) and added to the cell cultures in absence of antibiotics. *H. Pylori* was cultivated on TSA plates in microaerophilic conditions at 37° for 48-72 h. The infection was carried out in three different ways: (i) Competitive assay, in which AGS cells were incubated simultaneously for with *L. fermentum* and *H. pylori* for 2 h; (ii) Inhibition assay, in which AGS cells were preincubated with *L. fermentum* for 1.5 h and then *H. pylori* was added and incubated for 2 h; (iii) Displacement assay in which AGS cells were pre-incubated with *H. pylori* for 2 h and then *L. fermentum* was added and further incubated for 1.5 h. Finally, Real Time PCR was carried out to evaluate the expression of pro- and anti-inflammatory cytokines IL-6, IL-8, TNF-alpha, IL-1alfa, IL-1beta, TGF-beta and antimicrobial peptide human beta defensin-2 (HBD-2) with the LightCycler software. Results: the data obtained show that *L. fermentum* can almost completely inhibit the inflammatory state strongly induced by *H. pylori* in the displacement assay, by downregulating IL-1alfa, IL-6 and IL-8. Therefore, the probiotic is also able to increase the antimicrobial defenses by upregulating the expression of HBD-2, improving the conditions of the gastric mucosa damaged by *H. pylori*. Discussion and conclusions: considering that the treatments against *H. pylori* infections may cause serious side effects and increased antibiotic resistance, the use of *Lactobacillus* spp. instead of antibiotics could allow one to avoid this disadvantage.

137 - Induction of robust humoral immunity against SARS-CoV-2 both at post-infection and post-vaccine administration in a cohort of haematological cancer patients

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Induction of robust humoral immunity against SARS-CoV-2 both at post-infection and post-vaccine administration in a cohort of haematological cancer patients

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Impaired seroconversion upon SARS-CoV-2 infection has been repeatedly reported in "frail" patients, including patients with haematological malignancies (HM). To date, the effectiveness of the antibody response to SARS-CoV-2 following both infection and vaccination in patients with HM remains poorly understood. Here, we have analysed the humoral response to anti-SARS-CoV-2 in a disease-overarching cohort of 120 HM patients at two time points (post-infection and post-vaccination). In the study cohort the overall percentage of seroconversion, as judged by COVID-SeroIndex ELISA, at post-infection was 84.2% (101/120), which has increased to 94.2% (113/120) after vaccination. At both timings, the lowest values were found in patients with lymphoid malignancies or undergoing chemotherapy. Therapy-naive patients in the "watch and wait" status were more likely to seroconvert and display increased anti-s IgG titers. Quantification of neutralising antibody (NAb) levels by testing the sera against rVSV-SARS-CoV-2-$S_{\Delta 21}$ infection of Vero E6-TMPRSS2 cells revealed that, after infection, males, patients in the groups "watch and wait" or "complete/ partial response" during SARS-CoV-2 infection, and patients who experienced severe/critical COVID-19 all displayed enhanced neutralizing activity. Likewise, vaccinated patients included in the 'watch and wait' and 'complete/partial response' groups showed the highest neutralising titres. Furthermore, as observed also with anti-s IgG titres, no significant variations were found between single or double vaccine administrations. In both conditions, the proportion of patients with a neutralisation titre

sufficient to provide 50% protection from symptomatic COVID-19 always exceeded 50%, regardless of the HM type, but the percentage of protected individuals increased after vaccination, especially in patients with lymphoid malignancies.
Although our findings confirm the reduced seroconversion in HM patients, especially those with lymphoid disorders or undergoing chemotherapy-based treatment during SARS-CoV-2 infection, the overall neutralizing humoral response upon natural SARS-CoV-2 infection seems to be quite efficient and sustained overtime. Moreover, the humoral response after vaccine administration, even at a single dose, is significantly boosted in HM patients who recovered from SARS-CoV-2 infection with a percentage of patients displaying protection against severe COVID-19 falling in the range of 69 to 82%. Therefore, additional vaccine administration might be avoided, at least in the short-term period in this group of patients.

146 - THE VIRAL SENSOR IFI16 AT THE CROSSROADS BETWEEN DAMAGE AND TOLERANCE DURING HUMAN CORONAVIRUS INFECTION

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THE VIRAL SENSOR IFI16 AT THE CROSSROADS BETWEEN DAMAGE AND TOLERANCE DURING HUMAN CORONAVIRUS INFECTION

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Abstract

Unlike humans, bats can asymptomatically harbour different types of zoonotic viruses, such as severe acute respiratory syndrome coronaviruses (SARS-CoVs), thanks to their unique ability to efficiently regulate host immune responses to viral infections. Intriguingly, bats harbour genetic or functional loss of a number of proteins involved in innate immunity, including the PHYN gene family that comprises the interferon (IFN)-inducible gene IFI16. In humans, besides acting as viral DNA sensor involved in innate signalling and viral restriction, IFI16 appears to play a crucial role also in RNA virus infection. For example, IFI16 can bind to both influenza *viral RNA* and RIG-I, a pathogen recognition receptor (PRR), thereby leading to enhanced RIG-I-dependent IFN production and inhibition of viral replication. Fittingly, RIG-I has been recently shown to act as restriction factor in the early phase of SARS-CoV-2 infection. Thus, even though the cross talk between IFI16 and RIG-I during SARS-CoV-2 infection has not been analysed yet, it is very likely that both cellular receptors may play an important role in controlling human coronavirus (HCoV) replication and host innate response. Against this background, we hypothesized that IFI16 is a key regulator of PRR-mediated host response to HCoV infection in human epithelial cells and that therapeutic modulation of this pathway may impact HCoV replication/infectivity. To test this hypothesis, we used wild-type (WT) and IFI16 knock-out (KO) spontaneously immortalized human keratinocytes (HaCat) cell lines that were infected with two bat-derived HCoV e.g. the low pathogenic NL63 (alpha-hCoV) and the highly pathogenic SARS-CoV-2 (beta-hCoV). We have observed that infection of IFI16-depleted epithelial cells with the low-pathogenic virus NL63 leads to aberrant IFN production, which in turn interferes with the late stages of viral replication and the infectivity of the released virions. Similarly, SARS-CoV-2 infection is also impaired in epithelial cells lacking IFI16. Upon characterization of NL63 infection in a monkey epithelial cell line named LLC-Mk2, we have found that IFI16 trafficks from the nucleus to the cytoplasm in infected cells, whereby it colocalizes with the viral Nucleoprotein (NP), suggesting a potential interaction between IFI16 and NP or even with the viral genome. We are currently characterizing this interaction. Overall, the results so far obtained indicate that the IFI16 protein may interfere with the sensing of the viral genome with ensuing impact on the innate immune response during hCoV infection

149 - Correlations between chronic intestinal pseudo-obstruction (CIPO), gut microbiota and intestinal serotonin-related genes expression.

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Correlations between chronic intestinal pseudo-obstruction (CIPO), gut microbiota and intestinal serotonin-related genes expression.

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Introduction: Chronic intestinal pseudo-obstruction (CIPO) is a rare disease characterized by symptoms and radiological signs suggestive of intestinal obstruction, in the absence of lumen-occluding lesions. It results from an extremely severe impairment of propulsive motility. Serotonin (5-HT, a local mediator and neurotransmitter) release is linked to intestinal peristaltic and secretory reflexes. The intestinal microbiota and the enteric nervous system (ENS) interact and this results in effects on the synthesis of 5-HT, its release and the subsequent serotonin receptors' activation. The interplay between ENS/5-HT and dysbiosis in CIPO remains largely unclear. The project aim was to assess correlations between gut microbiota, intestinal serotonin-related genes expression and the disease status in CIPO pediatric patients. For this purpose, mucosa-associated microbiota (MAM) has been characterized, and changes in gastrointestinal (GI) serotonin pathway have been evaluated. **Material and Methods**: We collected biopsies of the colon, the ileum and the duodenum from 7 pediatric CIPO patients and 7 age-/sex-matched healthy controls at Cesare Arrigo Childrens' Hospital (Alessandria, Italy). After DNA extraction, the MAM was assessed by next generation sequencing (NGS) of the V3-V4 region of the bacterial RNA 16s, on an Illumina Miseq platform. The expression of genes implicated in serotoninergic pathway (*TPH1, SLC6A4, 5-HTR3* and *5-HTR4*) was established by qPCR, after total RNA extraction from the same tissue samples. Correlation analyses were performed to highlight relationships between MAM and the expression of genes linked to the production (*TPH1*), transport (*SLC6A4*) and reception (5-*HTR3*, 5-*HTR4*) of 5-HT, and clinical parameters of CIPO patients. **Results**: Our results revealed a MAM different in its composition and biodiversity with respect to controls. Network analysis evidenced, in CIPO patients, a microbial ecosystem with fewer species, less connected, and with a greater number of non-synergistic relationships compared to controls. qPCR analysis results revealed alterations, a general a decrease, in the expression of serotonin-related genes for CIPO patients when compared to controls. **Discussion and conclusion**: Results showed, for the first time in CIPO patients, a specific MAM associated to CIPO and an altered expression of genes related to intestinal serotonin pathway. A possible malfunctioning in the 5-HT pathway, maybe linked to or triggered by an altered microbiota, could be a mechanism underlying the intestinal motility disorder in CIPO patients. Our results could also represent the first step to design a new therapy targeting the microbiota or targeting the serotonin pathways, improving treatment outcomes and quality of life for CIPO patients.

150 - Characterization of citrullination during infection with DNA and RNA viruses: a new strategy for host-targeting antivirals drugs

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Characterization of citrullination during infection with DNA and RNA viruses: a new strategy for host-targeting antivirals drugs

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INTRODUCTION. One of the strategies devised by a number of bacteria and viruses to favor its replication consists in modifying host cellular proteins at the post-translational level, thereby altering their localization, interaction, activation and/or turnover. A post-translational modification (PTM) that is increasingly recognized to play an essential role in this regard is citrullination, also called deimination, a process where the guanidinium group of an arginine is hydrolyzed to form citrulline, a non-genetically encoded amino acid. This PTM is catalyzed by the calcium-dependent protein arginine deiminase (PAD) family of enzymes, which in humans is composed of five isoforms (PADs 1-4 and 6), with different tissue-specific expression and substrate specificities. Although aberrant citrullination has been detected in several inflammatory conditions, suggesting that it may play a pathogenic role in inflammation-related diseases, a direct correlation between citrullination and viral infections has only recently emerged. In the last few years our laboratory has defined the citrullination profile, the expression of the enzymes PADs and the citrullinated substrates in the course of infection with DNA and RNA viruses, Herpesvirus and Coronavirus respectively. In addition, we evaluated a large panel of PAD inhibitors for their antiviral activity against these virus classes in a wide variety of cell lines.
MATERIAL AND METHODS. We used Human Cytomegalovirus (HCMV) and herpes simplex virus type 1 (HSV-1) as models of DNA viruses and HCoV-OC43 and SARS-CoV-2 as models of RNA viruses. We tested the antiviral activity of well characterized PAD inhibitors. We used real time quantitative PCR to quantify copies of the viral genomes, Western blot analysis to evaluate the expression of PADs and viral proteins , and plaque assay to evaluate the production of new virions. Furthermore, we assessed the pattern of citrullination upon infection by using a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe.
RESULTS. Citrullinome analysis of infected cells reveals significant changes in deimination levels of both cellular and viral proteins, with interferon (IFN)-inducible protein IFIT1 being the most heavily deiminated one. Moreover, we show that in vitro IFIT1 citrullination impairs its ability to bind to 5'-pppRNA.

DISCUSSION AND CONCLUSION. In the present study, we unveil a signature of human Herpesvirus infection based on PAD-mediated citrullination of multiple cellular proteins to disrupt host defense mechanisms. Moreover, the pharmacological inhibition of PAD enzymes led to a significant reduction of viral replication.

151 - News Microbiological Marker in type 1 diabetes
The possible role of MAP And HERVs in the type 1 diabetes onset.

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News Microbiological Marker in type 1 diabetes
The possible role of MAP And HERVs in the type 1 diabetes onset.

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Introduction: Type 1 diabetes (T1D) is an autoimmune disorder, characterized by the production of autoantibodies to the pancreatic beta-cells. In previous studies, we indicated that *Mycobacterium avium subspecies paratuberculosis* (MAP) could be a potential risk factor for T1D, suggesting that MAP infection could induce immune imbalance. Emerging evidence has also indicated that Human endogenous retrovirus (HERVs) may play a role in etiopathogenesis of TD1. Indeed, the envelope proteins of HERV-W and HERV-K families have been detected in serum and pancreas of patients with T1D. In the present study, we investigated the role of MAP and HERVs in a Sardinian pediatric population with T1D by analyzing the humoral response and variation over time toward peptides derived from both pathogens. **Materials and methods**: Different immunogenic peptides were used to test sera reactivity: MAP3865c$_{125\text{-}133}$, MAP2404c$_{70\text{-}80}$ peptides homologous to host beta-cell antigens, HERV-W$_{93\text{-}108}$, HERV-W$_{248\text{-}262}$ and HERV-K$_{19\text{-}37.}$ Plasma samples collected from 69 patients with T1D, of which 26 at T1D onset, 23 from T1D diagnosed 1-5years, 20 with T1D diagnosed 6-12years, and 45 HCs were analyzed by indirect ELISA. To investigate the potential differences among the different antibodies in T1D patients, HCs we performed a Mann–Whitney test. The potential change in humoral response related to disease duration, the results of the four groups (onset T1D, 1–5years, 6–12years and HCs) were analyzed by Kruskal–Wallis test. **Results**: Statistical significative different response was observed in antibody titre against all MAP and HERVs peptides between patients at T1D Onset vs. HCs ($p<0,0001$). A statistically significative and progressive decline was observed in antibody level as early as the first year of the disease. On the other hand, this pattern occurred with all peptides but not for HERV-K which remains constant from the onset until the age of six years. **Discussion and Conclusions:** Our results support the hypothesis that both pathogens are linked to T1D etiology, and also that HERVs activation can be induced by certain infectious agent such as MAP. The results are in line with this hypothesis, given the high reactivity expressed in patients T1D Onset for all peptide fragments evaluated. The progressive decrease in antibody titre over the years could reflects the development of an active autoimmune process at the onset. A contribution of MAP in this process could result from molecular mimicry with homologous epitopes such as Zinc transporter 8 (ZnT8) and Proinsulin (PI), leading to autoimmune responses. Taken together, these findings support the hypothesis that MAP and HERV could act as risk factors for T1D, suggesting that they may serve as potential biomarkers.

153 - Peptide L18R plays an antimicrobial modulating action against polymicrobial biofilm in LCWB model

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Peptide L18R plays an antimicrobial modulating action against polymicrobial biofilm in LCWB model

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Abstract

Introduction: Chronic wound infections represent an important health problem for the reduced response to antimicrobial treatment of the pathogens organized in structured biofilms. Over the last few years, antimicrobial peptides (AMPs) of various origins have attracted great attention as potential anti-infective agents to face the spreading resistance to conventional antibiotics. This study evaluated the potential effects of the antifungal peptide L18R against the relevant chronic wound pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Materials and methods: The antimicrobial activity of L18R was evaluated against the single planktonic microbial populations, on single, dual and triadic species biofilms both in early stage and mature biofilm and in polymicrobial Lubbock Chronic Wound Biofilm (LCWB) model mimicking the spatial microbial colonization in a chronic wound. The evaluation of CFU, biofilm biomass detection, confocal and scanning electron microscopy analysis were performed. Results: L18R showed a significant antimicrobial activity against planktonic microorganisms (EC_{50} values ranging from 0.278 to 1.157 µM) and was able to differentially reduce the biomass of monomicrobial biofilms (it reduces early and mature biofilm biomasses of *C. albicans* of 97.19% ± 1.02 and 98.81% ± 1.68, respectively, and of *S. aureus* of 45.65% ± 1.53 and 57.37% ± 6.45, respectively. A slight reduction was observed on *P. aeruginosa*). No reduction of biomass was observed against polymicrobial biofilm. In mature LCWB, L18R caused a moderate reduction in total CFU number, with a variable effect on the different tested microorganisms. In particular, L18R expressed a modulate action against all detected microorganisms, in respect to the control (Amikacin) in which the reduction of bacteria growth was associated to a great increase of the yeast cells. These data were confirmed by live/dead confocal images. Discussion and Conclusions: The results obtained show a modulating action of L18R making it an ideal approach for skin wound healing and recommend further studies on its potential role as adjunctive therapeutic agent in chronic wound management also in association to conventional or alternative treatments.

154 - Potential preventive role of probiotics against Pseudomonas aeruginosa corneal infection

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Potential preventive role of probiotics against *Pseudomonas aeruginosa* corneal infection

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Introduction. The ocular microbiome plays an important role in the maintenance of physiologic homeostasis of ocular surface and in the prevention of ocular diseases. Alterations of the normal microbiome change microbial composition causing an imbalance in favor of pathogenic species. Among these, *Pseudomonas aeruginosa* represents a common cause of severe corneal ulceration. The development of new antibiotics for ocular diseases is very slow, highlighting the need for alternative non-antibiotic strategies. Among these strategies, the probiotic therapy could help restoring the homeostasis of ocular surface and preventing or reducing adverse ocular outcomes caused by infection. The aim of this study was to assess the preventive role of probiotics against *P. aeruginosa* infection on human corneal epithelial cells (HCE). Materials and Methods**.** The HCE were used to assess the extent of protection by probiotics against *P. aeruginosa* infection. Probiotics: *Lactobacillus reuteri* DSM20016, *Bifidobacterium longum* subsp. *infantis* DSM20088 and *Staphylococcus epidermidis* DSM1798. Pathogen: *P. aeruginosa* ATCC 9027. The following assays were performed: cytotoxicity and adhesion to HCE of probiotics after 24 and 48 h of contact. Antagonism, cytotoxicity, anti-inflammatory and antioxidant properties of probiotics against *P. aeruginosa* infection were evaluated as follow: pretreatment of HCE with each probiotic (5×10^7 UFC/ml) at 1 h and 24 h prior to *P. aeruginosa* inoculation (5×10^6 UFC/ml) followed by 1 h and 24 h of growth in combination. Results. *S. epidermidis* significantly increased HCE vitality and demonstrated a greater ability to adhere to them both after 24 h and 48 h of contact than other probiotics. None of the probiotics have been shown to have antagonistic activity against growth and adhesion of *P. aeruginosa* to HCE. Instead, they have been shown to restore significantly the HCE viability and to promote the anti-inflammatory and antioxidant activity when in combination with the pathogen. MTT assay revealed that the probiotic combination groups significantly increased cell viability compared to *P. aeruginosa* infection groups particularly when HCE were pretreated with each probiotic for 24 h and exposed to pathogen for the next 24 h. At the same conditions Elisa test showed that the pretreated HCE significantly reduced the levels of TNF-alpha and increased those of IL-10 whereas Griess assay significantly demonstrated reduction of nitrite/nitrate ratio. Discussion and Conclusions. These results show that pretreatment with probiotics has not a direct role player in contrasting growth and adhesion of *P. aeruginosa* to HCE but, is to be highlighted that it reduces key factors associated with *P. aeruginosa* infection.

157 - Prospective monitoring of HIV-1 infected patients after SARS-CoV-2 mRNA vaccination: characterization of IFN gene expression signature and humoral immune response

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Prospective monitoring of HIV-1 infected patients after SARS-CoV-2 mRNA vaccination: characterization of IFN gene expression signature and humoral immune response

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Background

COVID-19 vaccine has been reported to elicit humoral and T cell immune response in HIV-1 patients. Remarkably, some studies suggest that Interferon (IFN) signature might correlate with immunological and clinical efficacy of COVID-19 vaccines, as reported for Influenza vaccines. Understanding the early innate response to vaccine exposure and the humoral activation is needed to characterize mechanisms related to immunization in HIV-1 individuals. Therefore, the aim of this study was to evaluate the humoral response and IFN signature in HIV-1 positive individuals receiving COVID-19 vaccine.

Material and methods

Longitudinal effects of mRNA-based SARS-CoV-2 vaccine on humoral response in HIV-1 patients (n=82) were investigated measuring antibodies levels using LIAISON SARS-CoV-2 Trimerics IgG assay in serum samples collected before vaccination (T0), at the time of the second dose (T1), and 1 or 6 months following the second dose (T2). Expression level of mRNA encoding for distinct type I IFNs (i.e. IFN-Alpha2, IFN-Beta, IFN-Epsilon and IFN-Omega), and IFN-stimulated genes (ISGs), ISG15 and ISG56, were quantified by RT/Real time PCR assays in peripheral blood mononuclear cells (PBMCs) collected at T0, T1 and T2.

Results

Eighty-two HIV-1 patients (57 males (70%), 25 females (30%), mean age 56 years) on long-term ART were enrolled in this study. Results showed that humoral response following immunization increased significantly from T0 to T1 ($p<0.001$), and from T1 to T2 ($p<0.001$). Stratifying patients according to CD4 T cell count, no differences in antibody production were observed up to 1 month after the second dose. However, individuals with CD4 T cell count <400 cells/mm3 maintained a lower anti spike-antibody response 5 months after full vaccination compared to those with high

(>400 cells/mm3) CD4 T cell count ($p<0.05$). Gene expression analysis performed in 55 HIV-1 infected patients before and after COVID-19 vaccination, indicated an overall increased of IFN response up to 1 month after the second dose. Also, transcript levels of IFN-Alpha2, IFN-Beta, IFN-Epsilon and IFN-Omega, and of ISG15 and ISG56 were positively correlated with antibody production. By contrast, IFNs and ISGs mRNA levels decreased ($p<0.05$) at 5 months after the second dose, concomitantly with the reduction of antibody levels.

Conclusion

Our results confirmed that mRNA-based SARS-CoV-2 vaccine successfully promote antibody production in ART-treated HIV-1 patients and that the levels of CD4 T cells can impact on the rate of humoral immune response activation. Moreover, we demonstrated that transcript levels of IFN related genes changed in patients receiving COVID-19 vaccine according to the amount of anti-spike antibodies.

158 - Characterization of mucosal immune response against SARS-CoV-2 in vaccinated children.

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INTRODUCTION

Children generally develop mild or asymptomatic SARS-CoV-2 infection. Innate immunity has a key role limiting viral replication in the upper airway. However, the role of mucosal IgA in children protection is to be defined yet. For this reason, we measured salivary IgA in a group of SARS-CoV-2 infected children and compared with a group of vaccinated children.

METHODS

We enrolled 90 vaccinated children (VAX-C) consecutively admitted at the pediatric vaccine center of Umberto I hospital receiving BNT162b2 (Pfizer/BioNTech) from February to April 2022 and 41 children who had previous SARS-CoV-2 infection (INF-C) from October 2020 to March 2021 (age range 5-11 years). Serum and saliva of vaccinated children were collected on the day of 1st vaccine dose (T0) and 10 days after 2nd vaccine dose (T1) while samples from infected children were collected 20 days after first positive molecular test (T1). Anti-spike IgA and IgG in both serum and saliva were measured using ELISA assay (EUROIMMUN), saliva was diluted 1:20 and serum 1:100. Statistical analysis was performed with SPSS software v25.

RESULTS

About ten days after the second dose (T1), 59 children returned to follow up and we collected saliva samples and 21 serum samples. At T0, 28% of children already had either measurable or borderline values of anti-Spike IgA in the saliva. At T1 the rate of IgA positivity significantly increased ($p=0.023$) and the frequency of IgA positive samples increased to 49% whereas 51% of the saliva samples remained negative. Next, we stratified the results according to the children history of SARS-CoV-2 infection or close contact with infected family members. Eight children had experienced COVID 19 (8.8%), several months before vaccination (Infected-VAX) while 16.6% were considered exposed-VAX and 74.6% had no history of previous contact. Spike specific IgA significantly increased in response to vaccination in the naïve-VAX ($p=0.0019$), but not in exposed-VAX and infected-VAX. Salivary spike-specific IgG were not detectable before vaccination in all three groups, while at T1 34.8% were positive to IgG. Lastly, Serum IgA and IgG levels were significantly higher in vaccinated children compared to the INF-C group ($p=0.037$ and $p<10^{-8}$).

DISCUSSION

Our results showed detectable levels of salivary IgA and IgG in vaccinated children.

This finding is compatible with the transudation of vaccine-produced IgG from the plasma to the oral cavity. Measurement of secretory IgA levels in mucosa of vaccinated children in comparison with infected ones will further clarify mucosal response against SARS-CoV-2 induced by vaccination.

160 - Evaluation of redox state alterations in influenza A virus-infected neuronal cells: involvement in neurotropism.

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Evaluation of redox state alterations in influenza A virus-infected neuronal cells: involvement in neurotropism.

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Introduction: As viruses use cell machinery to replicate, multiple intracellular factors affect the outcome of viral infection; on the other hand, they induce considerable alterations in cellular homeostasis, as in the redox balance. Several redox modifications have been described during influenza A virus (IAV) infection in respiratory epithelial cells, but less is known about redox state in infected neuronal cells which, although not the main target, can be reached leading to nervous complications. Redox modifications in IAV-infected epithelial cells include: ROS production, glutathione (GSH) reduction, glutathionylated proteins formation, oxidoreductases (as glutaredoxins, Grxs) modulation and the up-regulation of matrix metalloproteases (MMPs), endopeptidases involved in the inflammatory process. Our hypothesis is that redox modifications could regulate neurotropism of some IAV strains, i.e. viral replication in neuronal cells and virus-induced inflammation. Therefore, the aim of this study was to analyse the role of the redox state in influenza virus replication and viral-induced inflammation in a neuronal model of infection.
Materials and Methods: Human neuroblastoma SH-SY5Y cells 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 by hemagglutinin assay and for the MMPs activity by gelatin zymography. Cell lysates were analyzed: for GSH content by GSH assay kit; for Grx1, MMPs expression by immunoblotting with specific antibodies. Proteins from infected and Glutathione ethyl ester biotin amide (BioGEE)-treated cells were run under non-reducing or reducing conditions and analyzed by western blot with streptavidin-peroxidase conjugated.
Results: NWS virus, that efficiently infects and replicates in SH-SY5Y cells, led to a reduction in free GSH intracellular level and an increase in glutathionylated proteins. Grx1 expression increased as well. In the supernatants of NWS-infected cells, we observed an increase in MMP2 activity. The treatment with a Grx1 inhibitor, 2-AAPA, that we already showed to reduce inflammatory cytokine IL-6 secretion from epithelial cells, reduced secreted MMP2 activity.
Discussion and Conclusions: The results describe redox alterations in NWS-infected neuronal cells, suggesting that redox-sensitive pathways could be involved in viral replication and virus induced inflammation in the nervous system.

163 - Fecal-associated Enterobacteriaceae isolates from Celiac disease patients: could dietary sugars drive changes in bacterial composition?

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Fecal-associated *Enterobacteriaceae* isolates from Celiac disease patients: could dietary sugars drive changes in bacterial composition?

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1. Introduction: Celiac disease (CD) is a multifactorial immune-mediated disorder of the small intestine triggered by ingestion of gluten in genetically predisposed individuals. Like several intestinal diseases (i.e., IBDs), CD could be associated with intestinal dysbiosis characterized by an increased abundance of pathobionts. Here, we aimed to characterize fecal-associated *Enterobacteriaceae* isolates from CD patients and assess possible effects of dietary sugars on bacterial community and pathogenicity. Active CD patients are treated with a strict gluten free diet (GFD); however, gluten-free foods are often high in carbohydrates content. Therefore, we wondered if sugars in GFD could increase the abundance of commensal/pathogenic gram-negative bacteria or decrease the abundance of beneficial bacteria. 2. Materials and Methods: Various *Enterobacteriaceae*-like colonies were isolated on selective and differential media (i.e., MacConkey and Chromogenic agar) from fecal samples of our cohort including active CD, CD patients under GFD and healthy individuals (n=25 for CD and GFD and n=18 for controls). Diverse colonies differing in size, shape, and colour were sequenced and identified at species level. The most abundant sugars found in the small intestine (i.e., mono, di- and polysaccharides) were used for a "sugar challenge" study. 3. Results: *Escherichia coli* was identified as the most abundant bacterium followed by non-*E. coli Enterobacterales* (NECE), such as *Klebsiella* spp. (i, e., *K. oxytoca, K. michiganensis*), *Enterobacter* spp., *Hafnia alvei/paralvei, Citrobacter freundii/koseri*, and *Serratia marcescens*. The total count (CFU/gram of feces) of *E. coli* was between 10 to 10^6 in CD and GFD vs 10^3 to 10^8 in controls), whereas NECE were enumerated between 0 to $<10^6$ among all groups. In some fecal samples, NECE were not isolated, being outnumbered by *E. coli.* The growth kinetics of the two most abundant isolated species (*E. coli* and *K. oxytoca* strains) were monitored either singularly or in co-culture in minimal medium supplemented with selected saccharides at different concentrations. We found that galactose, fructose and glucose differently boosted the growth rates of both species. Interestingly, *E. coli* outcompeted *K. oxytoca* in galactose-containing medium (1.8 to 2.4 log CFU/mL reduction), whereas *K. oxytoca* outcompeted *E. coli* in fructose-containing medium (0.8 to 1.4 log CFU/ml reduction). Mannose did not change any bacterial growth ratio. Neither *E. coli* nor *K. oxytoca* strains were able to grow in medium supplemented with sucrose at all concentrations tested. 4. Discussion and Conclusions: Overall, our results revealed that *Enterobacteriaceae* strains in fecal samples are highly heterogeneous among CD and GFD patients with respect to controls. Moreover, bacterial strains exhibited different sugar preferences to support their metabolism and growth. Pathogenic properties of CD-associated *E. coli*, determination of sugars in the feces through high performance liquid chromatography followed by complementary analyses at both genomic and proteomic levels are under investigation.

164 - Discovering the role of Mycoplasma girerdii in Trichomonas vaginalis pathobiology: just a parasite or a new friend for the protist?

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Discovering the role of *Mycoplasma girerdii* in *Trichomonas vaginalis* pathobiology: just a parasite or a new friend for the protist?

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Introduction: *Trichomonas vaginalis* is a protozoan pathogen of human urogenital tract causing the most common non-viral sexually transmitted disease worldwide. A fascinating feature of the protist is its capability to act in concert with endosymbiotic bacteria and viruses. Endosymbiosis involves two *Mycoplasma* species, *Mycoplasma hominis* and *Mycoplasma girerdii*, and up to four species of dsRNA *Trichomonas vaginalis* viruses. The influence of *M. hominis* on parasite's pathobiology and on dynamics of the host-parasite-bacteria interaction has been well studied, while the the influence of *M. girerdii* (a new non-cultivable *Mycoplasma* species) on *T. vaginalis* pathobiology is still largely unknown.

Materials & Methods: We developed an *in vitro* model system in which features of isogenic *T. vaginalis* strains with and without *Mycoplasma girerdii* were compared. The influence of bacterium on cytolytic and adhesive properties of the protozoon, and on its metronidazole sensitivity was evaluated. In addition, RNAseq experiments were set up to study the influence on *T. vaginalis* gene expression.

Results: *M. girerdii* increases the cytopathic activity of *T. vaginalis*, as well as previously demonstrated for the endosymbiont *M. hominis.* Moreover, its presence increases the *in vitro* capability of the protozoon to adhere to human cells. These results are further supported by transcriptomic data showing major shifts gene expression of metabolic enzymes and virulence genes in *T. vaginalis* infected by *M.girerdii* compared to the same protozoan strain lacking the bacterium. Interestingly, the presence of *M. girerdii* seems to have no effect on metronidazole resistance in *T. vaginalis*, while the presence of *M. hominis* significantly increases the sensitivity to metronidazole in trichomonad cells.

Discussion and Conclusions: Our data support the hypothesis that the presence of endosymbionts may modulate *T. vaginalis* virulence, reinforcing the idea to consider pathogenic entities as microbial ecosystems.

165 - Study of immunological parameters associated with Long COVID in children/adolescents

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Study of immunological parameters associated with Long COVID in children/adolescents

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Introduction

SARS-CoV-2 infection generally causes mild or no symptoms in children. Nevertheless, increasing evidence show that several immune-pathological changes can remain weeks or months after the infection; this persistent condition is known as "Long COVID (LC)", that in adults is associated to highly activated innate immune cells. In this context, interferon (IFN) response and inflammation-related miRNAs in peripheral blood mononuclear cells (PBMCs) could have an important role. Hence, we measured the expression in PBMCs of the type I IFNs (IFN-I) genes and of miR-141, miR-155 and miR-146a in children/adolescent with and without LC symptoms.

Materials and Methods

Blood samples from SARS-CoV2 (+) and (-) children attending Umberto I hospital of Rome, were collected within 3-6 months after SARS-CoV-2 tests. From PBMCs, RNA was extracted for determining cellular gene expression (IFN-I alpha2A, beta, epsilon and omega), and miRNAs expression by quantitative RealTime-PCR assays, normalized to housekeeping GUS gene and RNU6B, respectively.

Results

Ten SARS-CoV2(+) patients (mean age 8.5y SD 1.7) with LC symptoms, 10 SARS-CoV2(+) patients (mean age 9.1y SD 2.1) without LC symptoms and 10 SARS-CoV-2(-) children (mean age 10.8y SD 2.9) were enrolled. Comparing LC patients and patients that did not report LC symptoms, we found a downregulation of IFNalpha2A, IFNbeta and IFNepsilon ($p=0.016$, $p=0.016$, $p=0.010$, respectively) in LC group. Moreover, IFNalpha2A, IFNbeta and IFNomega ($p=0.041$, $p=0.010$, $p=0.016$, respectively) showed a lower expression level in LC subjects with respect to negative controls. We also observed an overactivation of miR-141 either in LC ($p=0.019$) or convalescents without LC ($p=0.001$) compared to negative ones. Contrastingly, miR-155 and miR-146a showed a tendency to be downregulated in LC as opposed to other groups.

Discussion and Conclusion

Overall, our findings reported a downregulation of IFN-I in LC subjects with respect to other groups analyzed. Conforming to several studies, IFN-I are activated during SARS-CoV-2 infection and diminish gradually after patient conditions improved. It is possibly to speculate that this waning trend could persist more during LC or, alternatively, that the lower levels we observed were due to a lower activation during the acute phase. Moreover, we observed an overexpression of miR-141 in SARS-CoV2 convalescents and LC children with respect to negative controls. According to recent investigations, miR-141 regulates NLRP3 expression and ultimately inflammasome. Taken together our findings would suggest that a dysregulation of the IFN response and a prolonged inflammatory state could lead to LC symptoms, in children; however, these preliminary data must be investigated further in larger cohort.

167 - Neutralization titers and Antibody Response to Heterologous Prime–Boost Vaccination with ChAdOx1 nCoV-19 and BNT162b2 in a Sardinian cohort group.

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Neutralization titers and Antibody Response to Heterologous Prime–Boost Vaccination with ChAdOx1 nCoV-19 and BNT162b2 in a Sardinian cohort group.

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Abstract: Introduction: Following several ChAdOx1 vaccine-associated adverse vascular events and thrombocytopenia, some countries recommended that patients under the age of 60 who received the first dose of ChAdOx1 receive a booster of the Pfizer BNT162b2 vaccine.

Material and methods: Quantitative IgG anti-SARS-CoV-2 Spike antibody (anti-S-IgG) and neutralization assay were run to investigate, in the long term period, the responses to vaccine treatment in our cohort of Sardinian subjects with heterologous priming with ChAdOx1 (ChAd) vector vaccine, followed by boosting with BNT162b2 (ChAd/BNT/BNT) and ChAd/ChAd/BNT). Results: The results were compared with those of a cohorts of health-care workers (HCW) who received homologous BNT162b2 (BNT/BNT/BNT) nCoV-19 vaccination or homologous regimen as a control group. At T2, one month after the second dose, and T3 (five months after the second dose), before the third dose, anti-spike antibody or neutralizing titers in the subjects given ChAdOx1-S/BNT162b2 were significantly higher than those given ChAdOx1-S/ChAdOx1-S or those given BNT162b2/BNT162b2.

Discussion and Conclusions: These data indicate that a ChAdOx1-S/BNT162b2 heterologous regimen provided a more robust antibody response than either of the homologous regimens. However, the anti-spike antibodies or neutralizing titers after the third injection (mRNA vaccine) of those given ChAdOx1-S as a second dose and those given BNT162b2 were not statistically different. Homologous and heterologous vaccination provided strong antibody response. Although the exact correlation between this response and protection has not yet been defined, subjects with a high neutralizing antibody response could be better protected against infection.

168 - CD169 IN BLOOD & CIRCULATING MICROVESICLES AS AN EARLY BIOMARKER OF COVID-19 DISEASE PROGRESSION

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Title: CD169 IN BLOOD & CIRCULATING MICROVESICLES AS AN EARLY BIOMARKER OF COVID-19 DISEASE PROGRESSION

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Introduction: The identification of early biomarkers for predicting COVID-19 progression and new therapeutic intervention for patient management are needed. CD169+ macrophages play an important role in viral infections and recently has been demonstrated that CD169 was strongly overexpressed in the blood of acute COVID-19 patients (A-COV). To investigate the possible association of CD169 with features of COVID-19 disease, we analysed CD169 in blood cells of A-COV at the time of hospitalization and correlated its expression with clinical characteristics and disease progression. Furthermore, based on recent work demonstrating the potential involvement of microvesicles (MVs) during SARS-CoV-2 infection, we characterised plasmatic MVs and assessed their potential role as a vehicle for the CD169 molecule in A-COV patients also in association with severity and characteristics of disease. **Materials and methods:** Flow cytometry was used for the evaluation of the median fluorescence intensity ratio of CD169 between monocytes and lymphocytes (CD169 RMFI) in blood samples from 27 healthy donors (HD) and 31 A-COV, and for immunophenotype and plasmatic MVs characterisation. **Results**: CD169 RMFI was significantly higher expressed in the macrophages of A-COV but not in those of HD and the ROC curve analysis show a high sensitivity and specificity value. In CD8+ T cells of A-COV, CD169 RMFI was associated with the decrease of naive cells and increase in Effector Memory cells. Finally, CD169 RMFI positively correlated with the senescence marker CD57+. Moreover, the CD169 RMFI increased with disease severity and pneumonia involvement in A-COV at sampling and reflects the respiratory outcome of patients during hospitalization. Flow cytometry analysis showed an increased percentage of MVS with a larger size (160-250 nm) in A-COV compared to HD. Interestingly, a significant increase in the Mean Intensity of Fluorescence (MFI) of circulating MVs expressing CD169 was observed in COV respect to HD. **Discussion and Conclusions:** Our data showed an association between CD169 expression and clinical status and respiratory outcome; moreover, the characterisation of MVs highlighted their contribution as a vehicle for CD169. Considering the immunological role of CD169 and its involvement during infection and COVID-19 progression, it could be considered an early biomarker of disease progression.

169 - Preliminary evaluation of genomic and transcriptomic changes in the evolution from a USA300 wild-type isolate to SCV, able to perform phagosomal escape.

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Preliminary evaluation of genomic and transcriptomic changes in the evolution from a USA300 wild-type isolate to SCV, able to perform phagosomal escape.

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Introduction

Small colony variants (SCV) of *Staphylococcus aureus* USA300 have been reported as implicated in chronic infections. We investigated through preliminary experiments that natural antioxidant carnosine is able to modulate the activity of macrophages infected with different *S. aureus* strains. In particular, by its well-known ability to ameliorate the energy state and the antioxidant machinery of macrophages, carnosine could be indirectly able to modulate the formation of SCVs by bacteria who have been able to perform phagosomal escape. Here, we investigated the genomic and transcriptomic changes involved in the evolution from a wild-type to a SCV form of USA300 strain in a Eukaryotic-Prokaryotic co-culture model.

Materials and Methods

The infection of RAW 264.7 cells was carried out with *S.aureus* USA300 with a multiplicity of infection (MOI) of 1:50. Extracellular bacteria were removed by lysostaphin for 1 hour at 37°C. After Carnosine treatment, macrophages were lysed, at different time points, and lysates were plated on Blood Agar in order to isolate internalized bacteria. RNA extraction was carried out for USA300 wild-type and SCVs. Quantitative real-time PCR RT-qPCR, was performed. GraphPad Prism 9 software was used for statistical analysis, p-values set up at > 0.05. NGS sequencing was performed by using Illumina DNA Prep and Illumina MiSeq. Data were analyzed using QIAGEN CLC Genomics Workbench software.

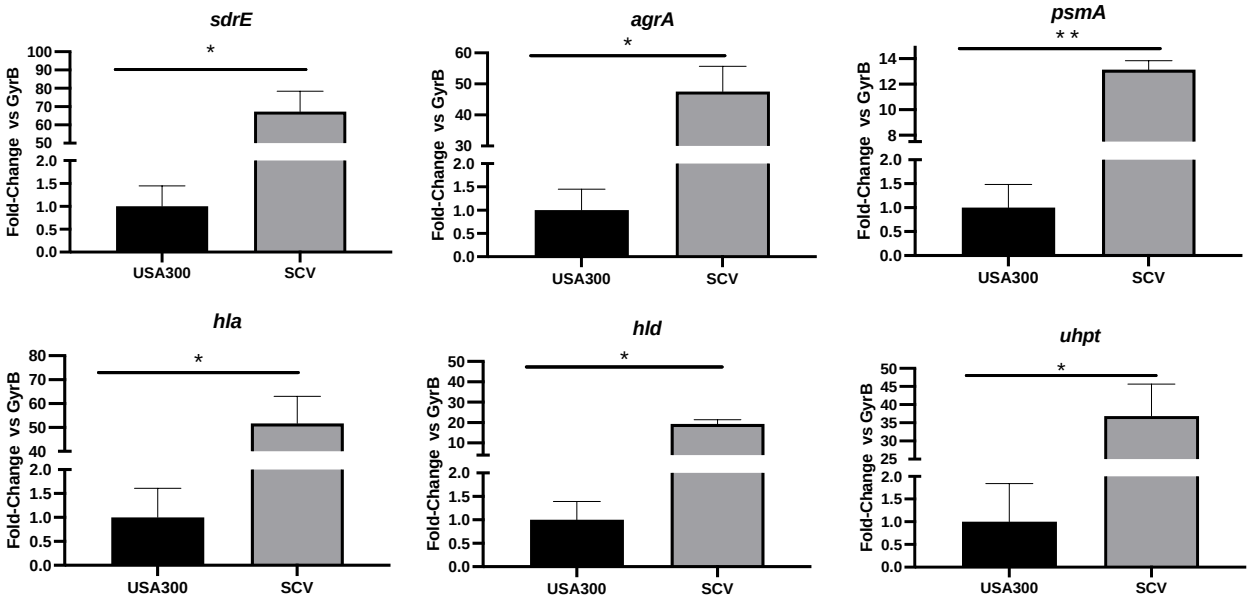
Results

Internalized bacteria, isolated after carnosine treatment condition, showed a SCV stable phenotype on Blood Agar. In particular, the SCVs, isolated 48 hours after the infection, were subcultured several times showing to be stable (no reversion to normal phenotype was reported). Preliminary Whole Genome Sequencing (WGS) analysis, revealed a mutation in SD-repeat containing protein D (*sdr*D), adhesin involved in interaction with host cells, belonging to the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Furthermore, consistent up-regulation of *sdr*E, *agr*A, *psm*A, *hla*, *hld* and *uhpt* was observed, as shown in figure 1, in the shift between colonization and invasive mechanism, suggesting a prominent role for these genes in staphylococcal pathogenesis.

Discussion and Conclusion

Our stable, SCV USA300 isolate seems to be a non-classical SCV phenotype, independent from the previously described hemin, thymidine or menadione auxotrophisms and dependent from an activated stringent response. Transcriptomic analysis highlighted a specific signature in the SCV strain including a complex, multi-level strategy of survival and adaptation to chronicity within the host, including a protection from the inflammatory response, an evasion of the immune response, and finally a constitutively activated stringent response.

Figure 1. Graph of quantitative real-time PCR RT-qPCR



t-Test Normalized Vs *girB*	*sdrE*	*agrA*	*psmA*	*hla*	*hld*	*uhpt*
P-value	0,0139	0,0149	0,0025	0,0241	0,0072	0,0289
95% Confidence Interval	32,38 to 100,2	21,8 to 71,4	9,52 to 14,73	16,25 to 85,21	11,57 to 24,98	9,05 to 62,65
P-value Summary	*	*	**	*	*	*

171 - In vitro analysis of epithelial tolerability and anti-Candida effect of a new lactic acid-based vaginal gel formulation

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***In vitro* analysis of epithelial tolerability and anti-*Candida* effect of a new lactic acid-based vaginal gel formulation**

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INTRODUCTION. Vulvovaginal candidiasis (VVC) is the most prevalent vaginal infection in adult women. It is mainly caused by *Candida albicans*, and it affects 75% of healthy women at least once during their reproductive age; 5-10% of such women have recurrent episodes (RVVC), with more of 4 episodes of acute VVC per year. Symptoms of VVC include itching, burning, swelling and redness of the vaginal mucosa with white vaginal discharge. The urinary system can also be affected, with pain and burning when urinating. This condition seriously damages the well-being and the life quality of the affected women. Since *Candida* is a commensal fungus of the vaginal mucosa of healthy women, the main question is how the fungus can switch from harmless component of the vaginal microbiota to virulent pathogen. In this work we analyzed the capacity of lactic acid-based vaginal gel formulation Respecta® Balance Gel (RBG) to counteract *C. albicans* virulence after epithelial cells infection *in vitro*.

MATERIALS AND METHODS. For the establishment of the *in vitro* infection model, we used a monolayer of the A-431 vaginal epithelial cell line and two different strains of *C. albicans* (strain SC5314 and the bioluminescent strain gLUC59). Dose-dependent experiments were performed to test the epithelial tolerability to RBG (IHS srl, Biofarma Group) by monitoring lactate-dehydrogenase (LDH) release from damaged cells. The capacity of RGB to counteract *Candida*-induced epithelial damage were analysed by monitoring LDH release from cells. Fungal growth and adhesion capacity during vaginal epithelial cells infection in the presence of RGB were evaluated by quantify the Relative Luminescent Units (RLU) and CFU counts, respectively.

RESULTS. Our results show that, at dilution 1:150, RGB is well tolerated by the vaginal epithelium and consequently we used this dose for the subsequent experiments. RBG was able to significantly reduce (by 65%) *C. albicans*-induced damage of vaginal epithelial cells. This effect was accompanied with the capacity of RGB to significantly reduce *Candida*

adhesion to the epithelium (adhesion reduction by 34%). Intriguingly, no inhibition of fungal growth was observed after 24h of infection in the presence of RGB in our experimental conditions.

DISCUSSION AND CONCLUSIONS. Our results show that RGB significantly reduces *C. albicans*-induced damage of vaginal epithelial cells. One of the mechanisms underlying this effect is the inhibition of *C. albicans* adhesion to the vaginal epithelial cells, which may prevent *Candida* from penetrating and damaging epithelial cells, hence counteract *Candida* virulence. Collectively our preliminary results suggest that RBG can strengthen the VVC therapy favoring the establishment of an ecosystem that prevent *Candida* virulence.

174 - Human endogenous retroviruses as markers of disease and prognosis of chronic lymphocytic leukemia

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Human endogenous retroviruses as markers of disease and prognosis of chronic lymphocytic leukemia

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Background: The dysregulated expression of human endogenous retrovirus K (HERV-K) at transcriptional and protein levels, as well as viral particle production, have been found in tissues, sera, and cell lines isolated from different types of tumors. In our previous studies, we have already demonstrated the concomitant expression of HERVs and embryonic genes in cancer cells with aggressiveness and stemness features. In the field of onco-hematology, some studies have identified alterations of HERV-K and others HERV families' expression in human lymphoid leukemic cells and the presence of anti HERV-K antibodies. Moreover, a few studies describe the potential involvement of embryonic genes in chronic lymphocytic leukemia (CLL). On this basis, the aim of the work was to evaluate the potential involvement of HERVs and embryonic genes in the etiopathogenesis of CLL and their use as biomarkers and prognostic markers of CLL. **Materials and methods:** Peripheral blood mononuclear cells were isolated from peripheral venous blood of 75 patients with CLL and 49 healthy donors (HDs). CLL patients were divided according to the treatment regimen they received. The expression of the ENV gene of HERV-K (HML-2), HERV-H, HERV-W, pathogenic HERV-W and HEMO, as well as the embryonic OCT4, KLF4 and NANOG genes and the stemness marker CD133 were analyzed by Real Time PCR. In addition, the percentage of HERV-K ENV-positive cells in B lymphocyte subpopulations (CD19+CD5+) was characterized by flow cytometry analysis. The genes expression profiles were defined by principal component analysis (PCA) in CLL patients, and their association with favorable prognostic factors of CLL was evaluated by multivariate analysis. **Results**: Expression profiles of HERVs and embryonic genes have been defined in CLL: the molecular analysis showed significantly higher expression of HERVs and embryonic genes in patients compared with HDs. Significant differences in all genes analyzed between untreated patients and those treated with chemotherapeutic or biological drugs patients were found. Moreover, a higher level of ENV HERV-K protein in CD19+CD5+ cells of CLL patients and its modulation in the presence of therapy were found. PCA revealed specific expression profiles of HERVs and embryonic genes in CLL patients, which were found to be associated with different treatment regimens and unfavorable prognostic factors. **Discussion and conclusion:** These results could help to clarify the CLL complexity, proposing HERVs and embryonic genes as potential new diagnostic and prognostic markers, suggesting their involvement in the etiopathogenesis of the disease. This scenario opens new avenues for investigating the use of HERVs and embryonic genes as potential therapeutics in combination with standard therapies.

178 - Maternal immune activation induces abnormal expression of endogenous retroviruses and immune effectors in a sex-dependent manner in a mouse model of Autism Spectrum Disorders

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Maternal immune activation induces abnormal expression of endogenous retroviruses and immune effectors in a sex-dependent manner in a mouse model of Autism Spectrum Disorders

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Introduction: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder resulting from complex interactions among genetic, environmental, and epigenetic factors. Maternal immune activation (MIA) is one of the major environmental risk factors recognized as increasing the odds of ASD in the offspring. MIA is triggered by infections and autoimmune diseases in the mother resulting in increased levels of cytokines and chemokines that cross the placental and blood-brain barriers affecting foetal neural development. A study conducted on two preclinical models of ASD, an idiopathic mouse model, and a mouse model induced by prenatal exposure to valproic acid, showed an altered expression of several endogenous retroviruses (ERVs) and cytokines, in embryos, blood and brain samples at different post-natal ages, supporting the potential involvement of ERVs in the pathophysiology of ASD. ERVs are relics of ancestral germline infections by exogenous retroviruses, stably integrated into the host cellular DNA, which are involved in the pathogenesis and progression of many human complex diseases. This work aims to study the expression of several ERVs families, ERV-related genes, immune effectors, and marker of damage to the central system (CNS), in several tissues of a well-validated mouse model of MIA, obtained with a single injection of viral mimetic analogous to a double-stranded RNA (Poly I:C). **Materials and methods**: C57BL/6J pregnant female mice were treated with Poly I:C or saline solution at gestational day 12.5. Behavioural evaluation of the offspring and tissue collection (pre-frontal cortex, hippocampus, and blood samples) were performed on post-natal day 60. The expression of several ERVs, ERV-related genes, proinflammatory and regulatory cytokines, toll-like receptors, and markers of CNS damage was evaluated by quantitative Real-Time PCR analysis. **Results**: The results obtained demonstrate that Poly I:C exposure modulates the expression of ERVs, ERV-related genes and inflammatory and regulatory cytokines in the pre-frontal cortex and hippocampus samples in mice showing autistic-like phenotype. Notable, the effect of Poly I:C is more marked in the pre-frontal tissue of female mice compared with males, while no statistically significant sex-dependent differences were found in hippocampus and blood samples. **Discussion and conclusions**: These findings support the hypothesis of the possible involvement of ERVs, ERV-related genes and immune effectors in the pathogenesis of ASD and demonstrate that prenatal exposure to Poly I:C can induce molecular sex-dependent differences in C57BL/6J mice with autistic-like phenotype.

182 - Effects of dysbiosis on 6-OHDA mouse model of Parkinson's disease: insights from core microbiota and co-occurrence network analyses

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Effects of dysbiosis on 6-OHDA mouse model of Parkinson's disease: insights from core microbiota and co-occurrence network analyses

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Introduction: In the last years, several studies highlighted the pivotal role of the gut microbiota as a key regulator of immunological and neuroendocrine mechanisms of the gut-brain axis in Parkinson's disease (PD). We propose a two-hit mouse model of PD in which ceftriaxone (CFX)-induced dysbiosis amplifies PD progression, worsening motor deficits induced by striatal 6-hydroxydopamine injection (6-OHDA) in mice. This effect is accompanied by a significant increase in neuronal dopaminergic loss, enhancement of systemic and colon inflammation, and worsening of colonic architecture. In this model we followed the dynamics of gut microbiota to infer a role for dysbiosis in exacerbation of PD symptoms.

Materials and Methods: Gut microbiota was studied by performing V3-V4 16S rDNA amplicon sequencing, then demultiplexed paired-end reads from MiSeq were processed and analyzed using the Quantitative Insights Into Microbial Ecology (QIIME2, version 2021.4). Structure, composition and network of interactions of gut microbiota were assessed by alpha and beta diversity, "qiime feature-table core-features" QIIME2 scripts, linear discriminant analysis effect size (LEfSe) method and Spearman co-occurrence network using the CoNet plugin for Cytoscape (3.9.0).

Results: Dual insulted mice showed a reduced gut microbiota richness with a core microbiota depleted of common gut colonizers, bacteria with probiotic activity, and butyrate producers, namely *Butyricicoccus pullicaecorum* and *Papillibacter cinnamivorans*. Microbial communities of 6-OHDA+CFX mice were also depleted in *Bifidobacterium breve* and *Alistipes finegoldii*, main acetate producers, suggesting the lack of cross-feeding interactions with the acetate-depending butyrate producers *B. pullicaecorum* and *P. cinnamivorans*. Network analysis built on LEfSe results indicated that the reduction of *A. furcosa, P. cinnamivorans* and *Defluviitalea saccarophila* drove the loss of the microbiota structure in 6-OHDA+CFX mice. Furthermore, *P. cinnamivorans, A. furcosa, B. breve*, and *A. finegoldii* were negatively correlated in the network analysis with *Ruminococcus lactaris*, a mucin-degrading commensal, strongly enriched in 6-OHDA+CFX mice.

Discussion and Conclusions: Our data suggest that the increase of *R. lactaris* in double insulted mice can compensate for the decrease of butyrate production bacterial networks by re-inforcing mucus glycoprotein degradation and that this activity could be detrimental to gut barrier integrity and promote pathophysiological traits of PD.

187 - Effects of antimicrobial protocols for Helicobacter pylori eradication on structure and functions of the human gut microbiota

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Effects of antimicrobial protocols for *Helicobacter pylori* eradication on structure and functions of the human gut microbiota

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1. Introduction
Antibiotic therapies are commonly used to eradicate bacterial pathogens. Despite their irreplaceable value, antibiotics cause an imbalance in the taxonomic structure of the gut microbiota as assessed by a number of metagenomics studies. However, no studies have yet described functional variations occurring in the gut microbiota in the context of antibiotic treatments.
The aim of our study was to investigate how antimicrobial standard protocols against *Helicobacter pylori* influence the human gut microbiota in taxonomic and functional terms, by profiling the gut metaproteome subjected to *H. pylori* eradication protocols, compared to the baseline.
2. Materials and Methods
Patients with *H. pylori* infection were prospectively recruited. Stool samples were collected at the enrollment, at the end and 30 days after the treatment. Gut microbiota composition and functions were analyzed using a metaproteomic approach. Protein extracts from samples were processed according to a modified filter-aided sample preparation (FASP) protocol and the peptide mixtures were analyzed through liquid chromatography-tandem mass spectrometry.
3. Results
The alpha diversity and the richness were calculated using data about peptide abundances. They decreased at the end of therapy compared with baseline and increased 30 days after the treatment. Studies at the level of peptide abundances allow a focus on microbial functions by highlighting different types of trends. First, there was a significant decrease following antibiotic therapy of certain functions, as bacterial *omega-amidase* and *aspartate-ammonia ligase* involved in amino acid metabolism. A second trend regarded functions significantly more represented immediately after antibiotic therapy and undergoing a decrease after thirty days, as in the case of the enzymes *glucose-fructose oxidoreductase* and *glucose-6-phosphate 1-dehydrogenase*. Finally, few functions including *Fe-S cluster assembly protein SufD* and *2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit beta*, were significantly different comparing baseline and thirty days after withdraw of therapy. These data were in agreement with peptide-based analyses of gut microbiota members that showed a change of taxa relative abundance according to the above-described trends.
4. Discussion and conclusions
Our study provides for the first time insight into the effects of antibiotic therapies on the functions of the human gut microbiota at metaproteomic level, confirming the resilience of most - but not all - gut microbiota members and of their metabolic functions after antibiotic therapy.

191 - HERVs IMPLICATION IN DEVELOPMENT, PROGRESSION AND LONG-TERM COMPLIANCE OF COVID-19

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Title: HERVs IMPLICATION IN DEVELOPMENT, PROGRESSION AND LONG-TERM COMPLIANCE OF COVID-19

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Introduction: The human coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated not only with elevated morbidity and mortality, but with long-term complications. Recent evidences highlight how the Human Endogenous Retroviruses (HERVs) were activated in response to infectious agents leading to immune-pathological effects and the present study aims to evaluate HERVs in the immune-pathogenesis of COVID-19, for the identification of new biomarkers for the diagnosis, prognosis, and follow-up of COVID-19 patients and their prioritization for targeted therapy. **Methods**: Blood samples were collected at Tor Vergata University Hospital of Rome from Acute COVID-19 (A-COV) patients and Healthy Donors (HD). Blood from Post COVID-19 (P-COV) was also collected (range: 7-48 weeks post-infection). The expression of the envelope (ENV) of HERV-K and HERV-W have been analyzed by flow cytometry and correlated with clinical signs, immunophenotyping, inflammatory markers, and disease progression. The T cell differentiation markers were also analysed. **Results**: HERV-W and HERV-K ENV proteins have been found expressed in blood samples from A-COV but not in HDs. Despite the percentage of Lymphocytes recovery, even after several weeks' post-infection the expression of HERVs remained elevated in P-COV. Among leukocytes, A-COV lymphocytes displayed the highest percentage of HERV-W ENV positive cells, which correlated with T cell differentiation, exhaustion, and senescence markers. Instead, HERV-K ENV resulted in highly expressed in granulocytes and directly correlates with senescence markers CD57. HERV-W ENV positive CD4+ T cells significantly correlated with coagulopathy and biochemical parameters associated with COVID-19 severity. In P-COV patients, HERVs protein expression remained high, especially in granulocytes. Notably, despite a restore of the normal distribution of leukocytes subpopulation in P-COV, the percentage of CD4 naïve cells and CD8 terminal effector memory resulted altered suggesting a persistent immune dysfunction in P-COV. To date, long-term health problems, including neurological symptoms such as headache, fatigue, dizziness, memory loss, confusion, and difficulty focusing, are associated with post-COVID-19 infection. Notably, HERVs expression was found higher in patients with specific neurological symptoms such as paraesthesia and tremors, suggesting their involvement in neurological alterations related to COVID-19. **Discussion and Conclusion**: These data suggest HERVs as contributing factors in the development, progression, and long-term complications of COVID-19 describing disease evolution and opening avenues for novel therapeutic strategies.

199 - Third Sars-CoV-2 vaccine dose: B cell and antibody responses following heterologous and homologous vaccination strategies

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Third Sars-CoV-2 vaccine dose: B cell and antibody responses following heterologous and homologous vaccination strategies

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Introduction: Since the end of 2021, many countries have implemented vaccination against SarS-CoV-2 with a third dose of the vaccine. Heterologous vaccine regimen has been a valid opportunity to make vaccination programs more flexible and reliable in the face of supply fluctuations and to improve immunogenicity and safety of COVID-19 vaccines. The available data on immunogenicity beyond one month following third doses are mostly related to antibody responses and T cell response while no information is available on the persistence of memory B cells that are expected to play a crucial role for a rapid response to SARS-CoV-2 infection. **Materials and Methods**: In this study, we profiled and compared the Spike-specific memory B cells, antibody responses and ACE-2/RBD binding inhibition activity following the administration of heterologous or homologous COVID-19 vaccines to 138 healthy volunteers without a laboratory confirmed history of SARS-CoV-2 infection. Subjects enrolled in the study were divided into three groups, vaccinated with different combinations of SARS-CoV-2 vaccines for the first vaccination cycle (first two doses) and for the booster (third dose), including the BNT162b2 (Pfizer-BioNTech; Comirnaty), mRNA-1273 (Spikevax, ModernaTX), and ChAdOx1 (AZD1222, AstraZeneca) vaccines. **Results.** The heterologous combination of ChAdOx1 (dose I and II) with mRNA (III dose) vaccines elicited Spike-specific IgG levels comparable with the ones induced by the three doses of mRNA vaccines. ACE-2/RBD binding inhibition activity was significantly increased in the heterologous and homologous vaccination schedule. A good antibody binding inhibition activity to SARS-CoV-2 Omicron variant was found in all groups. Ig switched S+RBD+ B cells were detected in all groups following third vaccine doses. The Ig switched S+RBD+ B cells significantly increased at +30 v3 compared to +7 v3 in heterologous vaccinations schedules while were maintained constant after homologous vaccination. A fraction of Ig switched S+RBD+ B cells showed a plasmablast phenotype (CD27+CD38high) at +7v3 and became almost undetectable at longer time points (+30v3). **Discussion and Conclusions** Heterologous prime-boost vaccination strategy elicits Spike-specific antibody and B cell responses comparable to the ones induced by homologous approach. These data indicate that heterologous prime-boost vaccination is an important strategy against SARS-CoV2 and other pathogens especially in state of emergency.

200 - Prediction of Pneumocystis jirovecii pneumonia in COVID-19 patients through quantitative PCR on oral washings

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Prediction of *Pneumocystis jirovecii* pneumonia in COVID-19 patients through quantitative PCR on oral washings

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INTRODUCTION. The impact of SARS-CoV-2 infection on the immune system, albeit not completely understood this far, has been widely described, and encompasses lymphopenia and functional exhaustion of immune cells. On the other hand, the use of corticosteroids for treating COVID-19 could have a similar effect, especially in the case of high doses or prolonged administration. This could constitute a substrate for the many different opportunistic infections reported in critically ill COVID-19 patients, which include *Pneumocystis jirovecii* pneumonia (PjP). However, the execution of bronchoalveolar lavages (BAL) to diagnose PjP is not always viable in these patients, suggesting the need for different strategies. The use of oral washings (OW) to assess *P. jirovecii* colonization has already been described on immune depressed patients. **MATERIALS AND METHODS.** Two different OWs were obtained from a COVID-19 inpatient: the first on the day of admission, the second twelve days later, when he also underwent a BAL for suspected PjP. DNA was extracted from OWs and subsequently underwent a real-time PCR targeting the mitochondrial large subunit ribosomal RNA gene of *P. jirovecii*, with a standard curve for quantitation purposes. The BAL was examined through a direct fluorescence assay (DFA) for *Pneumocystis* cysts and vegetative forms. **RESULTS.** The first OW was found positive for *P. jirovecii* DNA, at a concentration of about 25 copies/ml. The second was found negative. On the same day of the second OW, however, the BAL tested positive for PjP DFA. **DISCUSSION AND CONCLUSIONS.** This finding suggests that a diagnosis of *P. jirovecii* colonization in the oral cavity by the means of a real-time PCR on oral washings can anticipate by several days the detection of the same pathogen in profound respiratory samples in COVID-19 patients. Interestingly, *P. jirovecii* DNA could not be found in the oral cavity on the day it was found in the lower respiratory tract, suggesting the transition of the pathogen towards the lungs. Further expansions of this research could lead to a better understanding on the prevalence of *P. jirovecii* colonization and infection in COVID-19 patients and on the possible impact of preemptive therapy in selected cases.

202 - Human endogenous retroviruses as potential therapeutic targets for the treatment of head and neck squamous cell carcinoma resistant to radiotherapy

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Human endogenous retroviruses as potential therapeutic targets for the treatment of head and neck squamous cell carcinoma resistant to radiotherapy

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Introduction Human endogenous retroviruses (HERVs) are retrotransposon elements that originated from retroviral infections of the germ cell line millions of years ago. Altered expression of HERVs has been associated with the onset and acquisition of aggressiveness features in tumour cells. This study aimed to investigate HERVs as potential therapeutic targets for the treatment of radiotherapy-resistant head and neck squamous cell carcinoma. For this purpose, the effect of two reverse transcriptase inhibitors, efavirenz (EFV) and zidovudine (AZT), on the modulation of HERVs expression, the aggressiveness and survival of radioresistant (RR) and non-radioresistant (PAR) head and neck cancer cells (FaDu) was analyzed. Moreover, the effect of the inhibitor of a small GTPase, Rac1, in combination with antiretroviral drugs has been evaluated. Indeed, Rac1 is already known to regulate many cellular processes involved in radioresistance. Since radiotherapy is one of the most widely used strategies for the treatment of carcinomas, the effect of combined treatments was also evaluated during the exposure to ionizing radiation. **Materials and methods** FaDu-PAR and FaDu-RR cell lines were treated with antiretroviral drugs AZT and EFV, alone and subsequently in combination with Rac1 inhibitor (iRAC1) at different times. The relative expression of HERV-K (HML-2), HERV-H, and embryonic genes (OCT4, NANOG) were evaluated by Real-Time PCR. The expression of HERV-K ENV protein was assessed by flow cytometry. Colony formation assay and wound healing were performed. Moreover, the cell lines were exposed to ionizing radiation (2Gy,6Gy) after treatments to evaluate cell growth and apoptosis. **Results** FaDu-RR cell line showed high transcriptional activity of HERV-K, HERV-H, and embryonic genes OCT4 and NANOG, compared to the parental cell line. Treatment with antiretroviral drugs in combination with iRAC1 affected the transcriptional activity of HERVs and embryonic genes in FaDu-RR. Moreover, the co-treatment influenced clonogenic activity and cell motility. Furthermore, the co-treatment followed by exposure to ionizing radiations has induced the inhibition of cell growth and the induction of apoptosis in FaDU-RR cells. **Discussion and Conclusions** Nowadays, standard cancer treatments are often ineffective in carcinoma and new combination approaches are needed. The combination of antiretroviral drugs and iRAC1 was able to restore sensitivity to radiotherapy in FaDU-RR cells expressing high levels of HERV-K, suggesting its role in radioresistance and as a new potential therapeutic target.

205 - A specific anti-IFITM2 antibody bars the way to SARS-CoV-2 entry into host cells.

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TITLE

A specific anti-IFITM2 antibody bars the way to SARS-CoV-2 entry into host cells.

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ABSTRACT

Introduction

Viral proteins able to hook plasma membrane proteins/receptors give the start to complex cell processes leading to viral entry, replication and spreading. The early steps of viral infection involve protein complexes and structural lipid rearrangements, which mark the characteristic strategies of each virus on the way of its host cell. Innate immune response evolved first line defences aimed at restricting the viral entry and invasion, among them, human IFITM proteins are described as inhibitors of a broad range of viruses. Despite their homology and functional redundancy, recently, it has been surprisingly showed that SARS-CoV-2 is able to specifically hijack human IFITM2 in the infection mechanism of a wide range of host human cells. Here we report the synthesis and the characterization of the biological activity of a new and specific anti-IFITM2 mAb. Our study showed that the mAb is able to bind the extracellular N-terminus of the human IFITM2 and to prevent SARS-CoV-2 entry into the host cells.

Materials and methods:

A specific anti-IFITM2 mAb was obtained by immunizing mice with a specific antigen encompassing the IFITM2 extracellular N-terminal sequence. IFITM2 expression on SARS- CoV-2 permissive cells was detected by flow cytometry and the inibitory activity of the anti-IFITM2 mAb on the SARS-CoV-2 Spike protein entry was analyzed by Prossimity Ligation Assays. Finally, the antiviral activity of the anti-IFITM2 mAb was assessed by placque assay and by monitoring viral gene expression in RT-PCR.

Results:

Data supported the evidence of a strong expression of human IFITM2, as well as its monkey ortholog IFITM2/3, respectively on the cell membrane of SARS-CoV-2 permissive human cell line Calu-3 and monkey Vero E6 cells. Moreover, the use of this specific anti-IFITM2 mAb was able to impair the molecular mechanism leading to the endocytosis of the Spike protein. This effect was in agreement with functional assays showing that the IFITM2-blocking mAb was able to reduce SARS-CoV-2 dependent cytotoxicity and viral replication yield. These pieces of evidence let us infer that the antibody efficacy in reducing the success of the viral infection could be explained by its ability to interfere with spike internalization process.

Discussion and Conclusions:

The use of mAb against IFITM proteins can pave the way to the synthesis of new antiviral molecules as well as to a novel therapeutic strategy for the prevention, or early treatment, of Sars-CoV-2 positive patients. Because most emergency medications do not provide optimal responses, more valuable SARS-CoV-2 entry inhibitors are expected in the upcoming post-pandemic era, as well as in the context of counteracting new emerging viruses that spread through the respiratory tract.

209 - HSV-1 INDUCED COMPLEMENT ACTIVATION IN BRAIN CELLS: POSSIBLE TRIGGER OF SYNAPTIC DEFICITS

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HSV-1 INDUCED COMPLEMENT ACTIVATION IN BRAIN CELLS: POSSIBLE TRIGGER OF SYNAPTIC DEFICITS

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Introduction: Among the multiple factors that may contribute to the pathogenesis of Alzheimer's disease (AD), numerous experimental and epidemiological evidence suggests that recurrent herpes simplex virus-1 (HSV-1) infection reaching the brain is one of the risk factors for the disease onset and/or progression. However, the molecular mechanisms linking HSV-1 infection to neuronal dysfunctions have yet to be fully elucidated. Genetic, proteomic, and immunologic evidence suggests that dysregulation of the complement cascade, a key component of the innate immune system that is rapidly recruited to allow the clearance of pathogens and promote tissue repair, is involved in the pathogenesis of AD. It is also known that proteins of the classical complement pathway play a role in the process of synaptic elimination or neuronal pruning in the brain, an important event in neurodegeneration.

Materials and Methods: Primary neuronal cultures were isolated from the brain of rat embryos (E18). Neurons were mock- and HSV-1 infected at different multiplicity of infection and the efficacy of HSV-1 infection was evaluated by standard plaque assays (SPA). Cells were analysed for the expression of complement components and PSD95 (postsynaptic marker) at protein and mRNA levels with western blotting (WB) and RT-PCR, respectively. Immunofluorescence analyses (IF) were also carried to detect specifically C3 expression. Neutralization assay was performed with the aid of a specific antibody directed against the complement component C3.

Results: We found that HSV-1 infection in cultured rat brain cells induces an increase of C1q and C3 mRNA expression, detected by RT-PCR, and also a dose-dependent increase of C1q, C3, and C4 detected by WB. The C3 increase after HSV-1 infection was also confirmed by IF. Results from a neutralization assay showed that in HSV-1 infected neurons the inhibition of the C3 component rescued the HSV-1 induced decrease of PSD-95.

Discussion and Conclusions: The dose-dependent increase of complement proteins after HSV-1 infection in cultured rat brain cells indicates that complement may play a role in the immune response to HSV-1 infection in rat brain. The decrease of the synaptic marker PSD-95 expression after HSV-1 infection in cultured rat brain cells indicates a possible HSV-1 induced synaptic reduction. Finally, the preliminary results of C3 neutralization assay suggest that the complement protein C3 may be involved in the process of synaptic reduction that follows HSV-1 infection. Overall, these results suggest a possible complement-dependent synaptic damage triggered by HSV-1 brain infection, thus strengthening the causal link between HSV-1 and neurodegeneration.

211 - Antibacterial activity of Rhein against Streptococcus mutans

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Antibacterial activity of Rhein against *Streptococcus mutans*

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Introduction

Streptococcus mutans (*S. mutans*) is considered the major etiological agent of human dental caries and resides primarily in biofilms that form on the tooth surfaces, also known as dental plaque. The aim of our study was to evaluate the antimicrobial activity of a natural plant product, pure 4,5''-dihydroxy-anthraquinone-2-carboxylic acid (Rhein) against *S. mutans*.

Materials and Methods

Assay for antimicrobial activity and the time-killing test were performed to evaluate Rhein effects against planktonic *S. mutans*. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to determine the viability of immortalized human keratinocytes (HaCaT) following treatment with Rhein. The effect of different concentrations of Rhein on biofilm biomass and the metabolism of biofilm cells were evaluated through crystal violet and MTT assays. Further, Rhein-treated biofilms were viewed by confocal laser scanning microscopy. Rhein effects on acid production and acid environment tolerance were also assessed.

Results

The minimum inhibitory concentration (MIC) of Rhein, exerting bacteriostatic action on 90% of planktonic *S. mutans* (MIC_{90}), was 5.69 µg/mL. MIC and sub-MIC concentrations of Rhein affected the metabolism of biofilm cells and disrupted biofilm biomass with minimal biofilm eradication concentrations (MBEC) inducing 50% ($MBEC_{50}$) and 90% eradication ($MBEC_{90}$) of 6.31 and > 50 µg/mL, respectively.

Discussion and Conclusions

Confocal images displayed a significant reduction in biofilm biomass following treatment with increasing concentrations of the compound. Rhein also reduced the virulence of the biofilm by affecting acid production and acid tolerance.

Conversely, active concentrations of Rhein did not affect HaCaT cell viability. Together, these findings indicate that Rhein, a natural product that counteracts the virulence of *S. mutans*, may represent a novel therapeutic option for dental caries.

213 - Antibody and B-cell responses in myelofibrosis patients after the third doses of mRNA SARS-CoV-2 vaccines

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Antibody and B-cell responses in myelofibrosis patients after the third doses of mRNA SARS-CoV-2 vaccines

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Introduction. Vaccination against SARS-CoV-2 using mRNA-based vaccines has been highly recommended for fragile subjects, including myelofibrosis patients (MF). Available data on the immune response developed by MF patients after mRNA SARS-CoV-2 vaccination, and the impact of the therapy with ruxolitinib, are still fragmented. Here, we profile the spike-specific IgG and memory B-cell response in MF patients, treated or not with the JAK inhibitor ruxolitinib, after the second and the third dose of SARS-CoV-2 BNT162b2 (BioNTech) and mRNA-1273 (Moderna) vaccines. ***Materials and Methods.*** Plasma and peripheral blood mononuclear cells samples were collected at baseline (before vaccination), after the second (post v2) and third vaccine dose (post v3) and tested for spike-specific antibodies, ACE2/RBD antibody inhibition binding activity and Spike/RBD-specific B cells. The anti-spike IgG levels were assessed by ELISA. Memory Spike/RBD B cells were identified by multiparametric flow cytometry as $Spike^+$, RBD^+, $CD19^+$, $CD20^+$ and IgD^-**Results.** Seven days after two doses of mRNA SARS-CoV-2 vaccine, 76% of MF patients developed spike-specific IgG, but the response showed a slower kinetics compared to healthy subjects, suggesting a reduced capability of their immune system to promptly react to vaccination. A reduced ACE2/RBD binding inhibition activity of spike-specific antibodies was also observed, especially in ruxolitinib-treated patients. A two- and five-fold increase in spike-specific IgG was elicited by the third dose in patients with or without ruxolitinib treatment, respectively, while the percentage of subjects with antibodies capable of in vitro blocking ACE2/RBD interaction raised from 50% up to 80%, irrespective of the ruxolitinib treatment. A significant increase of spike/RBD-specific B cells was observed after the booster dose, especially in patients under ruxolitinib treatment, reaching values comparable with healthy controls. Overall, the third vaccine dose significantly enhanced the immune responsiveness in MF subjects except in three patients which were under immunosuppressive therapies at the time of vaccination. **Discussion and Conclusions.** Our results highlight a slow kinetic of the immune response in MF patients following the second vaccine dose of mRNA SARS-CoV-2 vaccines and show the capacity of the third vaccine dose of strongly boosting the spike-specific antibody and B-cell responses.

214 - Antimicrobial and Antibiofilm Activities of Carvacrol, Amoxicillin and Salicylhydroxamic acid Alone and in Combination versus Helicobacter pylori

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Antimicrobial and Antibiofilm Activities of Carvacrol, Amoxicillin and Salicylhydroxamic acid Alone and in Combination *versus Helicobacter pylori*

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Introduction. *H. pylori* eradication is difficult to be achieved because of its genetic variability, its capacity of forming biofilm and its ability of developing resistance against the commonly used antimicrobials. Antimicrobial resistance is a global health issue and the WHO specified *H. pylori* resistant to clarithromycin, as one of twelve high priority pathogens for which the antimicrobial research development is necessary. For these reasons, the identification of new strategies or adjuvants to standard antibiotic therapy, is required to counteract *H. pylori* infection. The inhibition of bacterial urease and carbonic anhydrases (CA) could represent a new mechanism of impairing the bacterial growth. The aim of this study was the evaluation of the antimicrobial and the antibiofilm activity of a CA inhibitor, such as carvacrol (CAR), amoxicillin (AMX) and a urease inhibitor, such as salicylhydroxamic acid (SHA), alone and in combination *versus H. pylori* ATCC 43504.
Materials and Methods. The Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal Concentration (MBC) of the combination of CAR-AMX, AMX-SHA and CAR-SHA were evaluated by the checkerboard method. The best MIC combinations of CAR, AMX and SHA were tested against *H. Pylori* mature biofilm. The ability of the combinations to eradicate *H. pylori* biofilm was evaluated through the AlamarBlue (AB) assay, the Colony Forming Unit (CFU) counting method and the Crystal violet assay.
Results. The compounds in combination were able to inhibit *H. pylori* planktonic growth, as demonstrated by the FIC index that is additive for the association of CAR-AMX and CAR-SHA but indifferent for the association of AMX-SHA. A preformed *H. pylori* biofilm was treated with double MIC concentrations of the compounds in combination and the results demonstrated that the most effective combinations are those with carvacrol. In particular CAR-AMX combination showed synergy in reducing the biofilm biomass already at 1xMIC concentration with complete eradication of the biofilm at 2xMIC. CAR-SHA association reduces *H. pylori* biofilm at 2xMIC concentration with a total eradication of the biofilm at 4xMIC. The association of AMX-SHA was the less effective in reducing *H. pylori* biofilm, in fact the complete eradication of the biofilm was reached at 4xMIC.
Discussion and Conclusions. The data obtained in this study showed that the combinations of CAR, AMX and SHA were able to eradicate a preformed biofilm developed by *H. pylori*, representing a potential innovative strategy against *H. pylori* infections. CA inhibitors and urease inhibitors may contribute, alone and in association with currently used antibiotics, to limit the spread of the antimicrobial resistance as well as the pathogenicity of this microorganism.

216 - Expression profile of Human Endogenous Retroviruses, their receptors, inflammatory and regulatory cytokines in children with Kawasaki disease and Multisystem inflammatory syndrome

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Expression profile of Human Endogenous Retroviruses, their receptors, inflammatory and regulatory cytokines in children with Kawasaki disease and Multisystem inflammatory syndrome

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Introduction: Kawasaki disease (KD) is a febrile systemic vasculitis which usually affects children younger than 5 years of age. The causative agent leading to the clinical manifestation in childhood remains unknown and the most widespread hypothesis is that an infectious factor triggers the excessive inflammatory response in genetically predisposed children. The recent Human Coronavirus Disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, highlighted the onset of a new post-infectious complication, called multisystem inflammatory syndrome in children (MIS-C), characterized by severe inflammation and clinical features overlapping Kawasaki disease. Human Endogenous Retroviruses (HERVs) are relics of ancestral germline infections by exogenous retroviruses, stably integrated into the host cellular DNA, which make up about 8% of the human genome. Deregulation of HERVs has been associated with many complex diseases, and recently our research group has demonstrated its involvement in the pathogenesis and progression of SARS-CoV-2-related disease. This work aims to study the expression of HERVs, their potential receptors and cytokines in blood samples of children affected by KD and MIS-C. **Materials and Methods**: 56 blood samples from affected children [4 with acute and 6 with subacute KD, 11 with acute and 13 with subacute MIS-C and 22 healthy children] were collected and the expression of several HERVs families (pHERV-W, HERV-K, HERV-H, HEMO, Syn-1, Syn-2), HERVs receptors (ASCT-1, ASCT-2, MFSD2A) and inflammatory mediators (IL-1□, IL-6, IL-10, IL-17, TNF-□, MCP-1, INF-□) have been analyzed by RT-Real time PCR. **Results**: The expression of pHERV-W and HERV-K was higher in children with MIS-C than in those with KD, reaching higher values in children with acute MIS-C. Higher levels of Syn-2 and its receptor MFSD2A were found in MIS-C patients than in KD, while the expression of Syn-1 and its receptor ASCT-1 was higher in all patients compared to controls. In children with MIS-C, the expression of IL-6 and TNF-□ was higher in the acute children group than in subacute, while the expression of IL-10 and IL-17 was higher in subacute children. IL-1□ expression was higher in all patients compared to controls. Finally, MCP-1 and IFN-□ expression was higher in children with acute KD than in the subacute group. **Discussion and conclusions**: These findings suggest HERVs a contributing factor in pathogenetic mechanism underlying KD and MIS-C and as potential molecular biomarkers.

217 - HSV-1-induced tau spreading in the brain: neurodegenerative damage or protective effect?

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HSV-1-induced tau spreading in the brain: neurodegenerative damage or protective effect?

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Introduction *G*rowing evidence supports the role of Herpes Simplex Virus-1 (HSV-1) infection in the pathogenesis of Alzheimer's disease (AD). In particular, HSV-1 replication induces the accumulation of AD-related neurotoxic proteins in neuronal cells, such as phosphorylated tau protein (ptau) and amyloid beta peptides (Abeta) both in monomeric and aggregated forms. These proteins can be released outside the cell both in a vesicle-free manner and via extracellular vesicles (EVs), likely contributing to the spreading of their dependent damage in the brain. Interestingly, recent data proposed Abeta as an antimicrobial peptide, whose production, aggregation and secretion could be induced in the infected cells to counteract the infection. Based on these findings, the aim of our study was to evaluate whether intracellular and secreted tau may exert, similarly to Abeta, a double role during HSV-1 neuronal infection, acting as an antimicrobial peptide and/or promote virus-induced neuronal damage.
Methods Primary neurons were isolated from embryo brains of wild type (wt) or tau KO mice. EVs were purified from supernatants of neuronal cells following HSV-1 infection (HSV-EV), treated with UV and layered on cells. Brain EVs were isolated by ultracentrifugation from cerebral tissues of HSV-1- and mock-infected mice. Cell lysates and EVs were analysed in WB for protein content. Levels of tau released in the supernatant were evaluated by ELISA assay. The efficacy of HSV-1 infection was evaluated by standard plaque (SPA) or in-Cell western (ICW) assays.
Results We found that HSV-1 infection induced the release of tau in the supernatant of infected cells both vesicle-free and via EVs. In particular, HSV-EVs contained increased amount of high molecular weight ptau with respect to EVs isolated from mock-infected neurons (ctr-EV). Accordingly, cells layered with HSV-EVs, previously treated with UV to inactivate the virus, showed higher levels of ptau, compared with ctr-EVs-treated cells. These results indicate that HSV-EVs could delivery neurotoxic proteins in uninfected neurons. Moreover, we found that HSV-1 viral replication was higher in primary tau KO neurons compared to wt neurons, suggesting that tau protein could interfere with HSV-1 replication in neurons. Finally, EVs enriched fractions isolated from the brains of HSV-infected mice contains several forms of ptau whose levels are modulated in HSV-1-infected mice compared with matched controls.

Conclusions Our results indicate that HSV-1 can promote ptau propagation, supporting the hypothesis that repeated HSV-1 reactivations may concur to neurodegeneration, but also suggest a potential protective role for tau against HSV-1 infection in neurons, highlighting a potential dual role of this protein.

218 - Antimicrobial and Antibiofilm Activities of Cell Free Supernatant of Limosilactobacillus reuteri DSM 17938

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Antimicrobial and Antibiofilm Activities of Cell Free Supernatant of *Limosilactobacillus reuteri* DSM 17938

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Introduction. The microbial biofilm has been defined as a "key virulence factor" for a multitude of microorganisms associated with chronic infections. Its multifactorial nature and variability as well as drug tolerance suggest the need to identify new compounds, alternatives to the commonly used antimicrobials. The Cell Free Supernatant (CFS) produced by many probiotic strains and containing many bioactive compounds such as biosurfactans, bacteriocines and antimicrobial peptides demonstrated the inhibition of the pathogens growth. However, each probiotic strain produces its own compounds.
The aim of the present study was to assess both the antimicrobial and subsequently the antibiofilm activities of Cell Free Supernatant (CFS) produced by *Limosilactobacillus reuteri* DSM 17938 versus biofilm-producer bacterial species of clinical relevance such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Fusobacterium nucleatum*, *Escherichia coli* and *Streptococcus mutans*.
Material and methods. The Minimum Inhibitory Concentration was determined through the broth microdilution method and cell proliferation assay; the Minimum Bactericidal Concentration by Colony Forming Units counts. The characteristics of the antimicrobial compounds were evaluated by pH adjustments, proteinase treatment and size fractionation of the CFS. The Minimum Inhibitory Biofilm Concentration (MBIC) and the Minimum Biofilm Eradication Concentration (MBEC) were determined through Colony Forming Units (CFU), Cristal Violet assay (CV) and a metabolic assay.
Results. The results showed (i) a greater efficacy of CFS towards Gram-negative compared to Gram-positive bacteria; (iii) a molecular weight <3 KDa as well as a non-proteinaceous nature of the antimicrobial compounds. The data obtained supported the hypothesis that the antimicrobial effect might be associated with a synergistic activity of several molecules or compounds contained in the CFS as previously hypothesized by other authors. In addition, the CFS showed a promising antibiofilm activity. In particular, among the tested bacterial species, the CFS showed a significative effect *versus P. aeruginosa* and *S. aureus* with a MBEC corresponding to 1xMIC. While, the MBEC values *versus E. coli* were 2-3×MIC. On the contrary, CFS *was not capable of inhibiting biofilm formation in none of the microorganisms tested.* The safety profile toward human cell lines showed promising sureness.
Discussion and Conclusion. The CFS of *L. reuteri* DSM 17938 might be useful for developing alternative therapeutic strategies against bacterial infections associated with biofilm-producer microorganisms. However, further studies should be performed todetect the CFS components responsible of the antimicrobial and antibiofilm activities.

233 - Longitudinal profiling of spike-specific antibody and B cell responses following mRNA-1273 SARS-CoV-2 vaccination in hematopoietic cell transplantation recipients

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Longitudinal profiling of spike-specific antibody and B cell responses following mRNA-1273 SARS-CoV-2 vaccination in hematopoietic cell transplantation recipients

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Introduction. Patients with hematologic malignancies treated with autologous (auto) and allogeneic (allo) hematopoietic cell transplantation (HCT) are at high risk of developing adverse outcomes related to SARS-CoV-2 infection. In the present work, we longitudinally profiled spike-specific antibody production and their functionality, as well as spike-specific B cell response in 23 auto-HCT and 56 allo-HCT recipients until the fourth dose of mRNA1273 vaccine. **Materials and Methods.** Blood samples were collected and analysed from baseline up to one month after the fourth mRNA1273 vaccine administration in HCT recipients enrolled in the context of a clinical study at the University Siena hospital. IgG serum antibodies were measured by ELISA, while the ACE2/RBD binding inhibition assay was performed as SARS-CoV-2 surrogate virus neutralization test. Spike-specific memory B cells were analysed by multiparametric flow cytometry. **Results. S**pike-specific IgG antibody production was observed in 90% of HCT recipients after the primary series of SARS-CoV-2 vaccination. The third and the fourth doses induced a robust increase in **s**pike-specific IgG and ACE2/RBD binding inhibition in all the HCT recipients, including the 10% of subjects that didn't respond to the primary series of SARS-CoV-2 vaccination. The booster doses also induced an increase in the spike-specific B cells that are crucial for a rapid response to SARS-CoV-2 virus encounter. **Discussion and Conclusions.** Our findings provide evidence that the mRNA1273 vaccine elicits strong spike-specific antibody and B cell responses in both allo- and auto-HCT recipients. The third and fourth booster mRNA1273 vaccine doses considerably enhance SARS-CoV-2 specific immune response highlighting the importance of additional vaccine doses in HCT recipients who may have achieved a limited response to the primary series of SARS-CoV-2 vaccination.

243 - Aspergillus infection and differential expression of TLRs and type I-III IFNs in the respiratory tract of a large cohort of CF patients from the Italian (Lazio) Reference Center for Cystic Fibrosis

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Introduction

Although an exceptional variety of fungal pathogens could be found in cystic fibrosis (CF) airway secretions, *Aspergillus* spp. is the main filamentous fungus cultured from the respiratory tract of CF airways with a prevalence range in CF patients between 10 and 57%. Clinical manifestations of *Aspergillus* infection are closely dependent on the immunological status of the infected host. In this scenario, given the pervasive influence of interferon (IFN) responses in the respiratory tract infections, it is imperative to characterize the potential involvement of type I-III IFN in the activation of immunity against *Aspergillus*.

Material and Methods

A total of 3746 search requests for *Aspergillus* research were processed from January 1st 2018 to December 31st 2019 on the respiratory samples of 467 CF patients attending the Regional Reference Center for Cystic Fibrosis, "Policlinico Umberto I". *Aspergillus* detection from respiratory samples was based on macroscopic and microscopic examination through culture on Sabouraud Dextrose Agar (BD Sabouraud). Gene expression of the most active toll like receptors (TLR) in fungal infections (TLR 2, 3, 4, 6, 8 and 9), type I and III IFN (IFNα2, IFNβ1, IFNε, IFNλ1, IFNλ2, IFNλ3), type III IFN receptor (IFNLR1) and the IFN stimulated genes 15 (ISG15) was performed by RT/Real Time-PCR assays on positive *Aspergillus* patients' samples (n=67) and on a matched group of *Aspergillus* negative CF patients (n=66).

Results

One hundred and thirty six samples (3.6%) of 83 patients (17.7%) were found positive to *Aspergillus* research request. A statistically significant increased expression of TLR3, TLR6 and TLR9-mRNAs was observed in the *Aspergillus* positive patients compared to the negative matched group ($p=0.009$, $p=0.044$, $p=0.001$). Moreover, higher IFNα, IFNβ, IFNλ2, IFNλ3 and IFNLR1 transcript levels were recorded in *Aspergillus* positive patients in comparison to the free ones ($p<0.001$, $p=0.006$, $p=0.048$, $p<0.001$, $p<0.001$). The first group presented higher expression of ISG15, a consolidated marker of IFNs' activation ($p=0.036$). Considering *P. aeruginosa* colonized patients, we found increased levels of ISG15,

IFNα, IFNε, IFNλ2, IFNλ3, IFNLR1, TLR3, TLR4, TLR8 and TLR9 genes ($p=0.002$, $p<0.001$, $p=0.017$, $p<0.001$, $p<0.001$, $p=0.009$, $p<0.001$, $p=0.002$, $p=0.001$, $p<0.001$) in *Aspergillus* positive CF samples compared to those negative for *Aspergillus* detection.

Conclusion

These findings suggest that *Aspergillus* colonization can increase the activation status of the IFN pathways in the airway tract of CF patients. Presence of both *Aspergillus* spp. and *P. aeruginosa* seems likely to determine a further increase of the IFN related immunity, suggesting that fungal-bacterial coinfection might contribute to alter airway innate immunity in CF patients.

254 - Evaluation of amphibian antimicrobial peptides as new therapeutic treatments against infectious diseases

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Introduction. In recent years, the resistance of pathogenic microorganisms to common antimicrobial agents is representing a severe public health problem. Moderate and wise use of antimicrobials and prevention of infections are the most effective methods for decreasing the spread and development of resistance. Therefore, also the COVID-19 pandemic has evidenced the urgent need for the discovery of broad-spectrum antiviral therapies that could be deployed in the case of future emergence of novel viral threats. Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs), represent an emerging class of therapeutic agents in different fields. In the present study, the antimicrobial activity of peptides derived from the secretion of the Rana genus has been evaluated against different human viruses and several bacteria pathogens for humans. **Material and methods.** AMPs have been 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, identifying a 50% cytotoxic concentration (CC50) at 50 μM. The antiviral activity was evaluated against different members of the *Herpesviridae*, *Paramyxoviridae*, *Coronaviridae*, *and Picornaviridae* (poliovirus, PV-1) families, through both plaque assays, molecular test, and Transmission electron microscopy (TEM) analysis. While antibacterial activity was investigated against several strains of gram-positive, gram-negative, and clinical isolates bacteria, identifying minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-killing assays. **Results**. AMPs did not show strong toxicity at the tested concentrations. When peptides were preincubated with viruses, significant antiviral activity was observed, demonstrating that they could disrupt the viral envelope, as also confirmed by TEM. The peptides influenced the extracellular phases of the viral lifecycle, probably by blocking the viral attachment and entry phases. They also showed a very strong antibacterial effect against all strains tested.**Conclusions.** Our results indicated possible novel applications of amphibian skin peptides in the field of antivirals and antibacterials. Further studies will focus on their specific mechanism of action to understand the antimicrobial target on which the peptides act.

264 - In vitro characterization of an mRNA vaccine model.

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In vitro characterization of an mRNA vaccine model.

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Introduction: In today's biotechnological landscape, mRNA vaccines represent a promising alternative to conventional approaches due to their versatility, safety, rapid production, and efficacy. In this project, a model mRNA vaccine was tested and characterized *in vitro* by transfecting it into different human cell lines.
Materials and Methods: The mRNA was obtained by an *in vitro* transcription reaction from a previously designed DNA template containing the gene of interest: Receptor Binding Domain (RBD) of Spike protein of SARS-CoV-2, with the ovalbumin epitope (OVA) and the influenza A hemagglutinin tag (HA) upstream and downstream, respectively. Transcribed mRNA was modified by enzymatic reactions, adding a 5' cap and a 3' poly(A) tail and then purified before transfection. Human epithelial cell lines were transfected with a complex of mRNA and lipofectamines as a delivery system and incubated for different time points. Transfected cells were analyzed by flow cytometric assay.
Results: Expression of heterologous protein was already detected after 6 hours and increased with time. After 24 hours of incubation, observations with an inverted microscope revealed the presence of detached cells in the wells containing transfected lipofectamine-RNA complexes. This phenomenon wasn't observed in control cells. Transfected cells stained with annexin V and propidium iodide confirmed apoptosis of the cells. Preliminary phagocytosis assays showed that apoptotic transfected cells were efficiently engulfed by THP-1 induced macrophage-like cells, compared with not transfected cells.
Discussion and Conclusions: An mRNA vaccine model was developed and characterized *in vitro*, and protein expression was detected by flow cytometry. In our model, detachment of transfected cells was observed after 24 hours, and apoptosis was studied. To get closer to *in vivo* conditions, phagocytosis assays were performed, which showed that only the transfected cells were engulfed. This suggested us to investigate the fate of the mRNA vaccines *in vitro* once they were injected into mammalian cells. The next step is to transfect the mouse myoblast cell line C2C12, followed by a phagocytosis assay using mouse macrophage J774A.1 cell line.

275 - SARS-CoV-2 vaccine immune response in adults with Down syndrome

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SARS-CoV-2 vaccine immune response in adults with Down syndrome

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Objective: People with Down syndrome (DS) are particularly vulnerable to coronavirus disease 2019 (COVID-19) and show altered immune response to vaccination. We aimed to evaluate the humoral and cellular immune response of a group of adults with DS treated with standard regimens of SARS-CoV-2 vaccine as compared with an age- and sex-matched group of persons without DS.

Methods: We compared antibody responses and cellular immune response between 42 subjects with DS (41.6 ± 10.8 years, 57% male), and an age- and sex-matched comparison group of healthy health care workers (HCW) (41.4 ± 8.8 years, 54.8% male) after SARS-CoV-2 vaccination with the standard regimen of BNT162b2 mRNA COVID-19 at 4 time points (baseline, 21 days after the first dose, 21 days after the second dose, and 21 days after the third dose). Receptor binding domain (RBD) IgG antibodies were assessed with Siemens SARS-CoV-2 IgG (COV2G) antibody test. We evaluated the specific cellular immune response assessing effector-regulatory T cells, B lymphocytes and monocytes subpopulations and specific for Spike peptide(s) and for SARS-CoV-2 stimulation.

Results: We observed significantly different antibody responses at all time points after vaccination (HCW vs. DS: 7.9 ± 3.9 vs. 1.4 ± 3.6 IU/mL at 21 days after first dose; 358.5 ± 3.8 vs. 38.1 ± 3.0 IU/mL at 21 days after second dose; 91.7 vs. 25.1 IU/mL at 21 days after third vaccination) and a significantly different time course of decline in antibody titers between the two groups. *In vitro* stimulation of circulating PBMC at different timepoints revealed an interesting modulation of specific cell subsets. Spike peptide or infection of PBMC culture with SARS-CoV-2 were not able to impact on B cells, probably for the potential low representation of circulating B lymphocytes specific for spike, corroborating the results obtained about Ab levels. The percentage of peripheral specific Th1 cells (IFNγ secreting $CD4^+$/Tbet+ T cells) was significantly reduced after second dose (p=0.004 T2 vs T0, and p=0.02 T3 vs T0). The vaccination did not impact on specific Th17 response in DS adults. Regulatory T cell compartment ($CD4^+$/$CD25^{high}$/$FoxP3^+$/$CD127^{low}$), although appeared significantly upregulated during the early phases after first dose

(p=0.02, T2 vs T0) was not anymore modulated by the second dose. Similarly the percentage of circulating M2 monocytes ($CD14^{+}/CD282^{-}/CD284^{-}/CD206^{+}/cd80^{-}$) significantly increased (p=0.04, T2 vs T0) during the early phases after the doses, while was reduced by the time.

Discussion: Subjects with DS have a valid antibody response to SARS-CoV-2 vaccination. However, this response is lower than that of subjects in the HCW group. This finding could indicate a more rapid decline in the protective effects of the vaccination in subjects with DS and could suggest that people with DS may benefit from a booster dose of vaccine.

277 - A predictive score of cancer immunotherapy responses based on ecological analysis of gut microbiota

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A predictive score of cancer immunotherapy responses based on ecological analysis of gut microbiota

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Introduction: Over the past decade, accumulating evidence points to the clinical impact of the intestinal microbiota on therapeutic outcome of various cancer treatments. Although specific gut microbial species have been associated with beneficial responses in meta-analyses, no consensus exists on a gut fingerprint identifying intestinal dysbiosis on an individual basis. **Materials and Methods:** Exploiting shotgun metagenomics sequencing of fecal samples from two retrospective cohorts of patients with advanced non-small cell lung cancer (NSCLC) prior to immune checkpoint inhibitors (ICI) initiation (n=393), we reconstructed ecosystem networks in responders and non-responders to ICI and detected patterns of bacterial species leading to a diagnostic tool predicting the individual response to immunotherapy. **Results:** The score was computed from topological networks of species interacting groups (SIG), identifying two SIG highly enriched in harmful (SIG1) or beneficial (SIG2) bacteria. Combining the SIG1/SIG2 ratio with *Akkermansia muciniphila* (Akk) abundance led to the TOPOSCORE, allowing estimation of the probability of a given individual to respond to ICI in a prospective cohort of 50 ICI-treated NSCLC patients with a superior accuracy than the actual prediction marker. The TOPOSCORE also predicted the response of 77 patients with advanced renal cell carcinoma treated with ICI. The sensitivity, specificity, positive and negative predictive value were 80%, 47%, 67% and 63%, respectively. The Cox proportional analysis revealed that the TOPOSCORE acted independently of and outperformed the actual clinical prognostic factors. Then, we exploited the publicly available datasets of MGS (n=641; NSCLC, RCC and melanoma) to validate TOPOSCORE across different cancer populations. Finally, we developed a PCR-assay measuring the prevalence of 7 harmful (SIG1), 16 beneficial (SIG2), and 1 discriminating (Akk) bacterial species. **Discussion and Conclusions:** TOPOSCORE represents the first easy-to-use and cost-effective tool capable of detecting intestinal dysbiosis associated with ICI resistance across cancers on an individual basis, under the precision-medicine egida. This TOPOSCORE has several implementations, to select donors and recipients of fecal microbiota transplantations and follow any microbiota-centered interventions.

285 - Epstein-Barr virus and Mycobacterium avium subsp. paratuberculosis homologues peptides elicit a strong humoral response in patients with neuroinflammatory disorders, and exacerbate EAE

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Epstein-Barr virus and _Mycobacterium avium_ subsp. _paratuberculosis_ homologues peptides elicit a strong humoral response in patients with neuroinflammatory disorders, and exacerbate EAE

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1. Introduction

Neuroinflammation can be induced by pathogens infection such as bacteria and virus, however, their pathological role still unclear. Here, we characterized antibodies against *Epstein-Barr* virus (EBV) nuclear antigen 1 (EBNA1), *Mycobacterium avium* subsp. *paratuberculosis* (MAP) heat shock protein (HSP) 70, and human central nervous system protein GlialCAM homologues peptides in blood samples of Japanese patients with different neurological disorders. Furthermore, we tested the effects of these peptides in active experimental autoimmune encephalomyelitis (EAE), a common animal model of neuroinflammatory disorders.

2. Material and Methods

Forty-seven patients with multiple sclerosis (MS), 32 with neuromyelitis optica spectrum disorder (NMOSD),19 with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), 32 disease controls, and 70 healthy controls, were retrospectively tested using indirect and inhibition ELISAs. Group of female C57BL6J mice received were immunized with the pathogen-derived peptides two weeks prior active EAE induction using myelin oligodendrocyte glycoprotein.

3. Results

A significantly strong antibody response against $EBNA1_{386-405}$, $MAPHSP70_{533-545}$ and $GlialCAM_{370-389}$ was detected in the serum of patients with MS and NMOSD in comparison to patient with MOGAD, and both control groups. A good direct correlation between antibody titers was evidenced in patients with MS and NMOSD. Moreover, competitive immunoassay demonstrated the presence of cross-reactive antibodies to EBV and MAP antigens. No significant correlation was found between antibody titers and clinical therapy, aquaporin-4 antibody titer.

Following immunization with $EBNA1_{386-405}$ and $MAPHSP70_{533-545}$ peptides, mice showed early onset and more severe disease in comparison to no treated mice.

4. Discussion and Conclusions

We demonstrated a strong humoral response and the significant presence of cross-reactive antibodies against EBV and MAP derived peptides in patients with MS and NMOSD, as well as the encephalitogenic potential of these peptides in EAE model, providing evidence for an association between these pathogens and different demyelinating disorders. Molecular mimicry could be the mechanism by which EBV, and MAP can induce autoimmunity in genetically susceptible individuals.

02 Meccanismi di patogenicità

11 - Streptococcal Surface Repeat (SSURE) domains from Streptococcus pneumoniae bind to different types of collagens

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Streptococcal Surface Repeat (SSURE) domains from *Streptococcus pneumoniae*

bind to different types of collagens

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Introduction: *Streptococcus pneumoniae* (Sp) is one of the main causes of community-acquired pneumonia. This pathogen is capable of efficiently adhering to host surfaces and invading respiratory epithelia by expressing specialized adhesins, including the Plasminogen and fibronectin binding protein B (PfbB). Here, we describe a PfbB-dependent mechanism whereby Sp promotes adherence to the extracellular matrix and invasion of host cells.
Materials and Methods: We examined encapsulated and unencapsulated Sp, including *pfbB* deletion mutants, for their ability to bind to different types of collagens (Coll) and to interact with cultured epithelial cells. We also used recombinantly expressed Streptococcal Surface Repeats (SSURE), that make up for ~80 of PfbB, and anti-SSURE antibodies.
Results: The absence of *pfbB* significantly decreased the capacity of Sp to adhere to collagens and C1q. Recombinant SSURE domains showed a remarkable degree of specificity in binding to different collagen types. For example, the C-terminal SSURE domain interacted with Coll I, II, III and the complement component C1q, while the N-terminal SSURE domain bound Coll IV and VI, but not C1q. Pretreatment of bacteria with C1q significantly increased bacterial adhesion to and invasion of epithelial and endothelial cells by acting as a bridge between PfbB and the $\alpha_2\beta_1$ integrin.
Discussion and Conclusions: Our data suggest that the SSURE domains of Sp play a significant role in the ability of this pathogen to interact with collagens, the most abundant constituents of the human body. These versatile domains also bind C1q that, as shown here, acts as bridge between the bacteria and the $\alpha_2\beta_1$ integrin expressed on host cells. Our results may be useful to develop new strategies to control Sp infections by preventing colonization and invasion of mucosal surfaces.

17 - Bio-guided approach in the search of novel antibacterial compounds from three Myrtaceae plants

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Bio-guided approach in the search of novel antibacterial compounds from three Myrtaceae plants.

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Introduction. Most current infectious diseases are almost untreatable by conventional antibiotic therapy given the rise in frequency of resistance among human pathogenic bacteria. *S. aureus* is a pathogen that can be part of normal human flora but can cause both community- and hospital-acquired infections. It can colonize almost every tissue and organs, as well implants and can manifest resistance to antibiotic therapy. The emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant *S. aureus*) is nullifying the efforts until now made virtually eliminating the use of B-lactams as therapeutic options against *S. aureus.* Myrtaceae (Juiss, year 1789) is an invaluable source of bioactive metabolites, such as terpenes, polyphenols and phloroglucinol derivates. These latter compounds have unique features and a wide range of biological and pharmacological properties especially antimicrobial activity, an attractive target for researchers. The current work aims to study *Myrcianthes cisplatensis*, *Psidium friedrichsthalianum* and *P. oligospermum* through antimicrobial assays and NMR analysis performed to identify new antimicrobial compounds.
Material and methods. Dried leaves of each plant, collected in Arizona, were extracted with solvents at increasing polarity: hexane, chloroform, and methanol. The obtained crude extracts were tested for their antimicrobial activity against two strains of *Staphylococcus aureus:* ATCC 29213 and 43300 (a methicillin-resistant *Staphylococcus aureus* strain, MRSA). NMR analysis was performed to characterize the bioactive fractions.
Results. Crude extracts in hexane and methanol of *M. cisplatensis* were the most promising, exhibiting a minimal inhibitory concentration (MIC) of 16 and 64 microg/mL against *S. aureus* ATCC 29213 and MRSA, respectively. Chloroform and methanol extracts of *P. friedrichsthalianum* showed MIC values of 64 and 128 microg/mL respectively against both the strains. On the contrary, *P. oligospermum* showed no activity. Starting from the methanol extracts, 1D- and 2D-NMR analysis allowed to investigate the bioactive fractions to have information about the metabolite composition. Three cinnamoylated alkylphloroglucinol glucosides besides coumarin derivatives, isolated from *M. cisplatensis*, and tetronic acid derivative from *P. friedrichsthalianum* were characterized by 2D NMR and tested for their antimicrobial potential.
Discussion and conclusions. The bio-guided approaches allowed the identification of new promising antimicrobial compounds from the bioactive crude extracts of *M. cisplatensis* and *P. friedrichsthalianum*. Deeper investigation will be focused to define the pure metabolite responsible for the antimicrobial activity and the identification of the molecular mechanism of action.

33 - Life cycle peculiarities of enteric Adenovirus 41

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Life cycle peculiarities of enteric Adenovirus 41

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Introduction: Adenovirus belonging to F species, 41 serotype (AdV41) represents one of the most important viral agents causing gastroenteritis in pediatric patients. Recently, the interest in AdV41 circulation and behaviour has intensified due to their postulated involvement in the sudden increase of hepatitis cases of unknown origin among children. The aim of this study was to evaluate the life cycle peculiarities of AdV41 by comparing it to AdV5, in order to investigate some of the possible mechanisms underlying their differential behaviour, focusing on cell cycle interference and apoptosis induction in connection with AdV E1 early gene functions.

Materials and Methods: Ten AdV41 strains were randomly selected among 118 AdV F identified from stool samples of paediatric patients admitted with acute gastroenteritis to the University-Hospital of Parma during the years 2010-2015. They had been analysed by conventional methods (electron microscopy and cell culture) for routine diagnosis purposes, identified by restriction endonuclease analysis as AdV41 and then stored at -80C°. AdV41 and AdV5 virus replication was evaluated by cell culture, immunofluorescence, electron microscopy and Real-Time PCR. Viral interference on cell cycle and apoptosis was studied by flow cytometry. E1 gene from both strains was sequenced.

Results: The results show that both serotypes were able to complete the lytic cycle, with lower effectiveness for AdV41. In contrast to AdV5, which has a typical cytocidal infection cycle, AdV41 gives rise to a non-lytic infection, even though it is able to complete its replication, producing a viral progeny which is able, in turn, to perform multiple infection runs with quite stable infection performance parameters. Moreover, a significant lack of cell accumulation into the S phase of the cell cycle was observed in AdV41 *vs* AdV5 infected cells, while no substantial changes in apoptosis levels were noted. Analysis of E1 gene coding regions evidenced relevant differences among the considered viral serotypes, mainly concerning the E1A region whose products are involved in viral replication and in the deregulation of cellular metabolism.

Discussion and Conclusions: These data support the ability of AdV41 serotype to give rise to non-lytic infections, possibly predisposing to viral persistence. This is of potential relevance considering that AdVs have been shown to persist in the gastrointestinal tract after primary infection in the pediatric setting, and that AdV41 could also reside in the human gut without causing disease, suggesting that it likely represents a risk factor for further development of intestinal or extra-intestinal pathologies.

52 - Serratiopeptidase affects adhesive features of Pseudomonas aeruginosa isolates from cystic fibrosis patients on biotic and abiotic substrates

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Serratiopeptidase affects adhesive features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients on biotic and abiotic substrates.

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Pseudomonas aeruginosa is an opportunistic pathogen often involved in airway infections of cystic fibrosis (CF) patients. During CF infection, the lung environment is extremely hostile because of the very high concentrations of antibiotics, reduced nutrient availability, elevated osmotic stress and intermicrobial competition: conditions that force *P. aeruginosa* to adapt for survival. The virulence of *P. aeruginosa* isolate is strongly related to different factors such as the capability to form a biofilm, different types of cell/colonial motility, production of toxins and the invasion of pulmonary cells. The dynamic process of biofilm formation offers protection to bacterial cells and resistance to drugs and host immune attacks. The self-produced exopolysaccharide matrix (EPS) that can incorporate different bacterial communities ensures their survival and resistance to certain antibiotics, complicating bacterial eradication. Motility also contributes to biofilm formation and bacterial colonization of surfaces. Furthermore, the ability to adhere is the prelude for the internalization into lung cells, a common immune evasion mechanism used by most intracellular bacteria, such as *P. aeruginosa*.

The impairment of bacterial cell adhesion and biofilm formation could represent a major target for the development of new therapeutic treatments for chronic infection control.

Previously reports evaluated the anti-infective properties of serratiopeptidase (SPEP), an extracellular metalloprotease produced by *Serratia marcescens*, in impairing virulence-related properties in bacteria, such as Staphylococci and *Listeria monocytogenes*. We demonstrated that SPEP was able to impair the attachment to inert surfaces and adhesion/invasion on eukaryotic cells with a mechanism independent by proteolytic mechanism.

This work aims to investigate the effect of SPEP on some virulence factors produced by *P. aeruginosa* isolated from CF patients, such as biofilm formation and accumulation, pyocyanin and pyoverdine production, motility and invasion to alveolar basal epithelial cells (A549 cell lines).

60 - Resveratrol Restores Susceptibility to Chlorhexidine and Benzalkonium in Microorganisms Causing Health-Care Associated Infection

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Resveratrol Restores Susceptibility to Chlorhexidine and Benzalkonium in Microorganisms Causing Health-Care Associated Infection

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1. Introduction

The spread of microorganisms causing health-care associated infection (HAI) is contributed by their intrinsic tolerance to a variety of biocides used as antiseptics or disinfectants. The natural monomeric stilbenoid resveratrol (RV) is able to modulate the susceptibility to chlorhexidine (CHX) and to benzalkonium (BZK) biocide in *Acinetobacter baumannii*. The aim of the study was to: (i) analyze the susceptibility to CHX and BZK biocides in a panel of reference strains and clinical isolates of Gram-negative bacteria, Gram-positive bacteria and yeasts; (ii) analyze whether RV may modulate and restore susceptibility to CHX and BZK in the above pathogens.

2. Materials and Methods

MIC (mg/L) and MBC (mg/L) values of CHX and BZK were determined by a broth microdilution method. In vitro combination studies were carried out using the checkerboard method. The combined effects were determined by calculating the fractional inhibitory concentration (FIC) index. Furthermore, the Pearson's correlation coefficient (p <0.01) was calculated both on CHX treatment in presence of the carbonyl cyanide m-chlorophenylhydrazine protonophore (CCCP) or RV and BZK treatment in presence of CCCP or RV.

3. Results

The CCCP efflux pump inhibitor reduced dose-dependently minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of CHX in 31 out 33 reference strains and reduced BZK MIC and MBC in 20 out 33 analyzed strains. RV from 32 to 256 mg/L decreased dose-dependently CHX MIC and MBC in 33 selected strains, while not restored CHX susceptibility in *Klebsiella pneumoniae* ATCC 700603, all *Pseudomonas aeruginosa* and *Candida* spp. strains. RV up to 128 mg/L decreased dose-dependently BZK MIC and MBC, but not restored BZK susceptibility in 3 *Burkholderia* spp., *Enterocobacter cloacae* ATCC 13047, *K. pneumoniae* ATCC 700603, *Stenotrophomonas maltophilia* K279 and all *P. aeruginosa* strains. Furthermore, CHX and BZK combination in the presence of 32 mg/L RV inhibited CHX and BZK MIC and MBC in all strains and restored CHX or BZK susceptibility in 16 out of 21 strains, resulting in a synergic or additive effect in 18 and 3 strains, respectively. However, CHX and BZK combination in the presence of RV at 64 mg/L restored CHX or BZK susceptibility in all strains and showed a synergic in 20 out 21 strains and additive effect in 1 out 21 strains.

4. Discussion and Conclusions

A synergic microbicidal effect was observed when the two biocides were combined with RV in a panel of Gram-negative bacteria, Gram-positive bacteria and yeasts.

RV reverts tolerance and restores susceptibility to CHX and BZK in the majority of microorganisms responsible for HAI. The combination of RV, CHX and BZK may represent a useful strategy to maintain susceptibility to biocides in several nosocomial pathogens.

63 - RiV4PoC: A high effective microbicidal coating for high-touch surfaces

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RiV4PoC: A high effective microbicidal coating for high-touch surfaces

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Introduction

Although airborne is the most efficient transmission route of various pathogens, the contamination of surfaces and objects remains a particularly effective path of indirect transmission. Infrastructures such as schools, airports, shopping centers and hospitals are particularly at risk, and specific attention must be paid to all those surfaces or objects that can function as a reservoir with a high load of microbes, even pathogens which represent a serious issue in hospitals where patients with weakened immune systems are more at risk than the general population. Naturally, the environment disinfection as well as hand hygiene represents the main tool of prevention of fomites transmission. However, this aspect is time-limited and difficult to control in public places. Therefore, it is necessary to develop technologies that have the ability to reduce or eliminate the microbes load on high-touched surfaces. Based on our solid previous data (patent WO2021255496A1), we aim to extend and strengthen the applicability of an innovative approach to limit the infectious load on contaminated surfaces of different materials in order to use it in the near future for medical devices and high-touch objects.

Materials and methods

We firstly performed structural, morphological, thermal and tribological characterization of the experimental model of coated surfaces, and then we applied the results of fundamental research to determine and validate the microbicidal activity of the coating against common contaminating bacteria *(*i.e., *Escherichia coli*, *Staphylococcus aureus*, *methicillin resistant S. aureus* [MRSA]) and viruses (i.e., alphacoronavirus 229E).

Results

In our experimental surface model, the coating formulation proved to have a potent bactericidal efficacy equal to 100% against Gram negative (*E. coli*) and positive (*S. aureus* and MRSA) bacteria. Moreover, the coating showed to be not toxic as evaluated by cytotoxicity assay and highly effective in reducing the initial infectious load of 229E by about 90% after exposure to the coating.

Discussion and conclusion

The main innovation of RiV4PoC is the revolutionary technological idea at the base which consists of a biocompatible, economical and future-proof formulation of a coating that exceeds the limits of toxicity/aggressiveness of the disinfectants on the market and which boasts an action which lasts over time. It will improve the health system resources with a direct impact on people's lives by effectively limiting the transmission of pathogens in the most risky social contexts. Such a tool would be of fundamental use in sensitive infrastructures (e.g. seats, handles, handrails) and commonly used devices (e.g. smartphones, medical devices) with enormous attractive potential.

70 - Characterization of stringent response phenotype in Neisseria meningitidis

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Characterization of stringent response phenotype in *Neisseria meningitidis*

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Introduction: Bacteria must sense and adapt to multiple changes that take place in their rapidly fluctuating environments. In response to starvation, the global regulators pppGpp/ppGpp (guanosine 5'-triphosphate or diphosphate 3'-diphosphate), collectively abbreviated as ppGpp, are the molecular effectors of the bacterial stringent response, an extensive reprogramming of transcription and metabolism. In detail, upon exposure to stresses (nutrient deprivation, antibiotics misuse) the increased concentration of ppGpp lead to the downregulation of proliferative processes (rRNA and tRNA synthesis, cell division) and the upregulation of stress response processes (amino acid biosynthesis and virulence gene expression) through the interaction of ppGpp with RNA polymerase (RNAP). Previously, through comparative transcriptomic analysis, we characterized a mutant rifampicin resistant (RifR) strain of *Neisseria meningitidis* serogroup C (93/4286 strain) with a substitution in 553 position (Men C H553Y) of *rpoB* gene cluster I for its ability to mimic stringent response associated to a peculiar downregulation of virulence genes. Here, we evaluated the stringent response phenotype in *N. meningitidis* serogroup C wild type strain (MenC) and its relative stringent-like mutant (MenC H553Y RifR) by the evaluation of kill curves against gentamicin, thiostrepton, ceftriaxone, ciprofloxacin and the assessment of main virulence genes expression, with and without an inductor of the stringent response. **Materials and Methods:** For the evaluation of killing curve, Men C and MenC H553Y RifR strains were incubated at 37°C with concentrations equal to the MBC of gentamicin, thyostreptone, ceftriaxone and ciprofloxacin and viable counts were assessed up to the sixth hour of contact. For Real time RT-PCR analysis, both strains, grown in minimal medium with and without DL-Serine hydroxamate (DL-Ser) a seryl tRNA synthetase inhibitor were evaluated for the expression of *opa*, *fhbp* and *pilE* genes. **Results:** The MenC H553Y RifR mutant showed a greater tollerance to the antibiotics acting on ribosomes compared to MenC strain; moreover, in presence of stringent response inductor, the expression analyses

revealed a downregulation of *opa*, *fhbp*, *pilE* genes in the MenC H553Y RifR mutant compared to MenC strain. **Discussion and Conclusions:** In line with the characterized stringent phenotype, the MenC H553Y-RifR mutant shown a greater tolerance to antibiotics acting on ribosomes and a downregulation of virulence genes probably mimicking the bacterial dormancy, a less obvious and perhaps even more widespread phenomenon that leads to antibiotic failure.

86 - Novel Options to Counteract Oral Biofilm Formation: in Vitro Evidence

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Novel Options to Counteract Oral Biofilm Formation: *in Vitro* Evidence

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1.Introduction: the oral cavity is a highly complex habitat, involving resident microbial communities importantly involved in local homeostasis. The biofilm production on biotic and abiotic surfaces is crucial step in the pathogenesis of most infections, particularly those occurring in the oral cavity. Its prevention and/or control may promote oral health and facilitate the management of patients with oral diseases. In this study, the antibiofilm activity of a biomimetic hydroxyapatite and a natural compound, MicroRepair (MicroR) and pomegranate (PomeGr), respectively, was assessed.
2.Materials and Methods: By luminescence/fluorescence-based assays, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* (*C. albicans*) were tested for their biofilm production in the presence of MicroR and/or PomeGr.
3.Results: we found that that microbial exposure to PomeGr resulted in a marked decrease in *P. aeruginosa* biofilm formation. A partial, but non-significant, reduction was observed with *S. aureus*, while a significant impairment occurred in *C. albicans* exposed to PomeGr. Differently, the MicroR treatment reduced biofilm formation in all the strains tested, especially in *C. albicans*, where the reduction was highly significant ($p < 0.0001$).
4.Discussion and Conclusions: by mean of *in vitro* models, the antibiofilm activity of a biomimetic hydroxyapatite, MicroRepair, and the natural compound, pomegranate, has been established. Given the crucial role of biofilm formation in the pathogenesis of many oral diseases, our results offer a basic rationale for the design of trials aimed at maintaining health conditions or recovering homeostatic imbalance in the oral cavity.

143 - A novel phenolic derivative inhibits AHL-dependent Quorum Sensing signaling in Pseudomonas aeruginosa

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A novel phenolic derivative inhibits AHL-dependent Quorum Sensing signaling in *Pseudomonas aeruginosa*

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Introduction: Increasing antibiotic resistance and declining pharmaceutical industry's investments have amplified the need for novel treatments for multidrug-resistant microbes. Quorum sensing (QS) inhibitors reduce pathogens' virulence without selective pressure on microbes and provide an alternative to conventional antibiotic-based therapies. *P. aeruginosa* (PA) uses complex QS signaling to control virulence and biofilm formation. We aimed to identify inhibitors of *P. aeruginosa* QS acting on lactones-mediated circuits.
Methods: Bioluminescence and qRT-PCR assays were employed to screen a library of 81 small phenolic derivatives (used at 50-100 µg/ml) for the ability to reduce AHL-dependent signaling. Cytotoxicity was then evaluated on procaryotic and eucaryotic cells by growth curve and MTT respectively. On PAO1 culture, treated or not with the most active compound, we determined: 1) AHL production by liquid chromatography plus mass-spectrometry analysis; 2) the biofilm disruption by resazurin method; 3) the production of pyocyanins, elastase, and rhamnolipids release by colorimetric method 4) adhering bacteria on pulmonary epithelial cells A549 by CFU counting 5) inhibition of interleukin-8 release by A549. Moreover, we collected 20 PA clinical isolates from patients with respiratory tract infections; elastase and pyocyanins were evaluated in treated or not treated isolates. By *in-vivo* assay on *Galleria mellonella* larvae, we evaluated the compound's ability to protect from PAO1 infection. Finally, by molecular docking, we examined the interaction between phenolic derivatives and QS receptor LasR or RhlR.
Results: We identified GM-50 as the most active compound inhibiting the expression of AHL-regulated genes but devoid of cytotoxic activity in human epithelial cells and biocidal effects on microbes. GM-50 reduced virulence factors such as rhamnolipids, pyocyanin elastase secretion, and swarming motility in PAO1, a PA laboratory strain. By molecular docking, we provided evidence that GM-50 highly interacts with RhlR. GM-50 significantly improved aztreonam-mediated biofilm disruption. Moreover, GM-50 prevented adhesion of PAO1 and inflammatory damage in A549 cell line and protects *G. mellonella* from pathogen-mediated killing. Additionally, GM-50 significantly reduced virulence factors in the 20 PA clinical isolates.
Conclusions: GM-50 inhibits AHL-signaling, reduces the expression and production of virulence factors, and enhances the anti-biofilm activity of aztreonam. It protects *G. mellonella* larvae and human A549 cells from damage induced by PA. Since GM-50 is active on clinical strains, it represents a starting point for identifying and developing new phenolic derivatives acting as QS-inhibitors in PA infections.

170 - Role of the histone deacetylase Hst3p in Candida albicans biology

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Role of the histone deacetylase Hst3p in *Candida albicans* biology

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1. Introduction. Infections caused by the polymorphic fungus *Candida albicans* are considered a serious concern either for the emerging drug resistance but also for the limited therapeutic options, that often target human cells due to their affinities to fungal cells with undesirable outcomes. Alternative strategies are needed to overcome the limitations of the current therapeutic plan. Many studies revealed that among various chromatin modifications, histone acetylation-deacetylation takes a leading role in *C. albicans* pathogenicity and virulence. In yeast, histone H3 Lys56 acetylation (H3K56ac) is an important post-translational modification that contributes to fungal genome integrity and depends on the activity of two opposite enzymes, the histone acetyltransferase RTT109 and the histone deacetylase Hst3p, a sirtuin inhibited by nicotinamide (NAM). Considering that, *HST3* deletion leads to decreased cell viability, genomic instability and attenuated virulence, and that fungal Hst3p family members share sequence motifs that are uncommon in human sirtuins, inhibition of Hst3p could be an attractive potential target for antifungal therapy. **2. Material and Methods.** To assess the effect of Hst3p on cell morphology, *C. albicans* cells treated with 10 mM NAM were observed by optical microscopy. In order to identify H3K56ac enriched regions across *C. albicans* genome, yeast cells treated as above were used for Chromatin immunoprecipitation assays followed by sequencing (ChIP-seq). Furthermore, to identify which pathway is altered by Hst3p inhibition, *Candida* trancriptome was analyzed by RNA-sequencing under the aforementioned conditions. **3. Results.** Our data show that Hst3p inhibition promotes the accumulation of H3K56 acetylation, which leads to the formation of an abnormal and enlarged filamentous structures with a particular V conformation (V-shaped hyphae) associated to a dysregulation of genes involved in adhesion, hyphal growth and cell wall maintenance.

176 - Skin dysbiosis in inflammatory acne lesions and the role of Cutibacterium acnes biofilm

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Skin dysbiosis in inflammatory acne lesions and the role of *Cutibacterium acnes* biofilm

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ABSTRACT

Introduction. Acne vulgaris is a common inflammatory disorder affecting more than 80% of young adolescents. *Cutibacterium acnes* plays a role in the pathogenesis of acne lesions, although the mechanisms are poorly understood. **Materials and Methods.** The study aimed to explore the microbiome at different skin sites in acne patients and the role of biofilm production in promoting the growth and persistence of *C. acnes* isolates. Swabs from non-inflammatory (NI) and inflammatory lesions (LA) of 10 acne patients and the skin of 10 healthy subjects (HS) were analyzed using 16S rRNA sequencing. In addition, traditional culture methods were combined with whole-genome sequencing. **Results.** Microbiota analysis showed a significantly lower alpha diversity in LA than in NI and HS. Differences at the species level were driven primarily by the overabundance of *C. acnes* on LA than NI and HS. The phylotype IA1 was more represented in the skin of acne patients than HS. Genes involved in lipids transport and metabolism, as well as potential virulence factors associated with host-tissue colonization, were selectively detected in phylotype IA1 strains. Additionally, the IA1 isolates were more efficient in early adhesion and biomass production than other phylotypes showing a significant increase in antibiotic tolerance for ampicillin, benzylpenicillin, clindamycin, and doxycycline. **Discussion and Conclusions.** Our data indicate a site-specific dysbiosis in LA compared to HS. Dysbiosis in the LA microbiome and colonization by virulent and highly tolerant *C. acnes* phylotypes may explain the prevalence of acne in a part of the population, despite the universal carriage of the microorganism.

179 - Genotypic and phenotypic characterization of Staphylococcus aureus strains isolated in patients with atopic dermatitis

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Genotypic and phenotypic characterization of *Staphylococcus aureus* strains isolated in patients with atopic dermatitis

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Introduction: Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease. The interplay between impaired barrier integrity, genetic disposition, immune system imbalance and microbial dysbiosis plays a key role in AD development. On the inflamed skin of AD patients, an alteration of the bacterial diversity with an increased *Staphylococcus aureus* proliferation has been observed. It has been evidenced that *S. aureus* produces several virulence factors, it can form biofilms in AD lesions and it is able to invade and survive in keratinocytes, contributing to the severity and progress of the disease. Furthermore, although specific *S. aureus* strains were most frequently identified in AD patients, no specific clone prevailed in this group of patients. So, the main purpose of this study is the identification of *S. aureus* lineage-specific features associated to AD.

Materials and Methods: *S. aureus* isolated from AD patients, sampled at flare (t0) and post-flare (t1) phases, and from healthy carriers were identified by MALDI-TOF and genotyped by RAPD-PCR. Whole genome sequencing analyses of *S. aureus* strains, including multilocus sequence typing (MLST), *spa* typing, Agr typing, detection of genes associated to virulence factors and antibiotic resistance, core genome MLST, were performed. The antimicrobial susceptibility test (AST) was determined using Vitek-2. Biofilm production was detected through crystal violet staining. The *S. aureus* invasion ability was evaluated by *in vitro* gentamicin protection assays on human keratinocytes (HaCat cells).

Results*:* At t0, *S. aureus* was recovered in 100% of lesional skin samples and at t1 in 35.7% of skin ones, with a higher degree of colonization at t0 than at t1. RAPD-PCR analysis revealed a clonal diffusion within the host during disease flare, with a same strain that can persist in the post-flare. Molecular typing evidenced a heterogeneity of strains, from both AD patients and healthy carriers, confirmed by the phylogenetic analysis in which all *S. aureus* strains tended to cluster regardless of the origin of isolation and of the disease. AST revealed a sensitiveness to a wide range of antibiotics in most strains, and no MRSA strains were detected. Moreover, *S. aureus* strains from AD patients were strong or moderate biofilm producers, while those from healthy carriers were moderate or weak biofilm producers. Finally, most *S. aureus* strains from AD patients were non-invasive.

Discussion and Conclusions: No specific genomic and functional features of *S. aureus* strains associated to AD were identified, suggesting that *S. aureus* strains shared a strong and similar pathogenic potential, that they express in a susceptible host and in particular environmental conditions.

197 - Molecular characterization of hypermucoviscous and hypervirulent strains of Klebsiella pneumoniae: an emergent health care threat

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Molecular characterization of hypermucoviscous and hypervirulent strains of *Klebsiella pneumoniae*: an emergent health care threat

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Introduction. In recent years, *Klebsiella pneumoniae* become a serious infections agent resulting from its increasing resistance to antibiotic therapy. Furthermore, recently, additional genetic traits associated with hypervirulence and antibiotic resistance have been identified that make *K. pneumoniae* infection an increasingly emergency problem. The aim of this study is to characterize 19 isolates of *K. pneumoniae* identify as hypermucosviscous phenotype, focusing on the clonal relationship and through various genes linked to virulence factors.

Materials and methods. In order to identify strains of *K. pneumoniae* hypermucoviscous is applied the string test considered positive with a length > 5 mm. All strains were subjected to MLST (Pasteur protocol). The production of biofilm was also analysed through the cristal violet test perform on microtiter plate. The identification of hypervirulent strains were performed searching the following genes: *iroB, iucA, rmpA* and *rmpA2* and *peg-344*, which correspond respectively to the loci of Salmochelin siderophore, Aerobactin siderophore, hypermucoidy and alleged transporter. The *magA*, a gene encoding a component of K1 capsule formation, was also detected through PCR. Carbapenem resistance was investigated through Carba test NP and positive strains has been subjected to specific PCRs for the bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{OXA-48} genes.

Results. 19 *K. pneumoniae* strains with a positive string test were investigated for hypervirulence factors. All 19 strains resulted strong biofilm producer. 7 strains out of 19 showed the presence of at least four of the hypervirulence genes screened and according to the literature we considered as hypervirulent *K. pneumoniae* (hvKpn). Two more strains showed the presence of *rmpA2* gene and we considered as potential *hvKp* pathotype. *magA* gene related to hypermucoviscosity was found in 6 strains out of 19 and just in one case in the presence of other virulence factors. Regard to MLST 3 out of 7 hvKpn strains were ST65, two ST29. Other important ST found were ST383, ST307 and ST101. Carba test NP established that only 4 out of the 19 strains have the enzyme responsible for the hydrolysis of carbapenems and carbapenemases were detected in different combination: KPC alone, OXA-48 alone, KPC and OXA 48, and one strains (ST383) harboured VIM. NDM and OXA-48. The latter is considered also a potential pathotype since it harbours the *rmpA2* gene, while the other three strain harbouring carbapenemases did not showed the hvKpn pattern.

Conclusions. Positive string test could be used to select strains forfurther investigations on virulence factors related to hvKpn pathotype. We detected hypervirulent genes in almost 50% of the string test positive strains. This phenomenon must be monitered and further studies are necessary to understand better virulence factors related to hvKpn. ST already related to hypervirulence were found, like ST65 (3 strains out 7). Note of worthy is the report of a ST 383, characterized by the co-production of OXA-48, VIM and NDM-5. This could represent a new hypervirulent XDR *K. pneumoniae* clone, as recently reported in northern Italy.

274 - PGRS domain structures: doomed to sail the mycomembrane

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PGRS domain structures: doomed to sail the mycomembrane

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Abstract

The impact of artificial intelligence in understanding biological processes is potentially immense. AlphaFold predicted the structure of thousands of proteins, including the enigmatic PE_PGRS protein family of *Mycobacterium tuberculosis*. Structural elucidation of mycobacterial PE_PGRS obtained with AlphaFold offers a unique opportunity to discuss how the findings obtained on these structurally complex proteins can be illuminating to solve key questions in biology. Gathering previous experimental findings and hypothesis with the predicted structures, we propose a PGRS “sailing” model as a smart tool to quickly move across the mycomembrane, for shipping enzymes or for host interactions.

03 Virologia

4 - Competition for dominance within replicating quasi-species during prolonged SARS-CoV-2 infection in an immunocompromised host

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Competition for dominance within replicating quasi-species during prolonged SARS-CoV-2 infection in an immunocompromised host

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Introduction: Since the beginning of the pandemic, different Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) variants, defined as a variant of concern (VOC), have arisen worldwide. It has been suggested that the emergence

of VOCs has possibly occurred in immunocompromised patients who allowed intra-host persistent infection and variations in viral population. The RNA virus population consists of an ensemble of replicating viruses characterized by closely related sequences termed quasi-species, allowing RNA viruses to have a greater possibility to find the best adapting quasi-species to the specific host. However, the underlying mechanism leading to the emergence of VOCs during viral persistence in the immunocompromised host is still unknown. Here, we describe the existence of an ensemble of minor mutants in the early biological samples obtained from an immunocompromised patient and their dynamic interplay with the master mutant during a persistent and productive long-term infection. **Materials and methods:** RNA was extracted from nasopharyngeal swabs (NPSs), libraries were generated and sequenced on Illumina MiSeq platform. Viruses were isolated from NPSs and used to perform *in vitro* assays. **Results:** We were able to follow the dynamics of the intra-host evolution during a SARS-CoV-2 prolonged infection in an immunocompromised patient. After 222 days of active viral replication the original master mutant, named $MB61^{0}$, was replaced by a minor quasi-species ($MB61^{222}$) expressing two critical mutations in spike, namely Q493K and N501T. Isolation of these two viruses allowed us to show that $MB61^{222}$ entry into target cells occurred mainly by the fusion at the plasma membrane (PM), whereas endocytosis characterized the entry mechanism used by $MB61^{0}$. Co-infection of human cell lines with the SARS-CoV-2 isolates highlighted the predominance of $MB61^{222}$ over $MB61^{0}$ replication. This finding may be explained by the capability of $MB61^{222}$ to induce peculiar viral RNA sensing mechanisms leading to an increased production of Interferons (IFNs) and, in particular, of IFN-induced transmembrane protein 1 (IFITM1) and IFITM2. **Discussion and Conclusion:** On the whole, our analysis points to a crucial role of SARS-CoV-2 quasi-species in intra-host evolution aimed at achieving the best fitness to adapt to the human host. Our results propose that during SARS-CoV-2 infection host innate immunity exerts a selective pressure on quasi-species, leading to the establishment of the SARS-CoV-2 master mutant able to escape host innate responses. This finding may explain the rapid worldwide turnover of VOCs that use the PM fusion pathway to enter into target cells over the original pandemic strain.

6 - IFI16 impacts metabolic reprogramming during human cytomegalovirus infection

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IFI16 impacts metabolic reprogramming during human cytomegalovirus infection

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Cellular lipid metabolism plays a pivotal role in human cytomegalovirus (HCMV) infection, as increased lipogenesis in HCMV-infected cells favors the envelopment of newly synthesized viral particles. As all cells are equipped with restriction factors able to exert a protective effect against invading pathogens, we asked whether a similar defense mechanism would also be in place to preserve the metabolic compartment from HCMV infection.

Here, we show that gamma interferon (IFN-gamma)-inducible protein 16 (IFI16), an restriction factor able to block HCMV DNA synthesis, can also counteract HCMV-mediated metabolic reprogramming in infected primary human foreskin fibroblasts (HFFs), thereby limiting virion infectivity. Specifically, we find that IFI16 downregulates the transcriptional activation of the glucose transporter 4 (GLUT4) through cooperation with the carbohydrate-response element-binding protein (ChREBP), thereby reducing HCMV-induced transcription of lipogenic enzymes. The resulting decrease in glucose uptake and consumption leads to diminished lipid synthesis, which ultimately curbs the *de novo* formation of enveloped viral particles in infected HFFs. Consistently, untargeted lipidomic analysis shows enhanced cholesteryl ester levels in IFI16 KO versus wild-type (WT) HFFs.

Overall, our data unveil a new role of IFI16 in the regulation of glucose and lipid metabolism upon HCMV replication and uncover new potential targets for the development of novel antiviral therapies.

19 - Mesenchymal stem cells are susceptible but non-permissive to B19V replication

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Mesenchymal stem cells are susceptible but non-permissive to B19V replication

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Mesenchymal stromal cells (MSCs) are multipotent stem cells with high expansion potential and self-renewal capacity. Their main characteristic is the ability to differentiate in vitro into multiple lineages, including osteocytes, chondrocytes, adipocytes and endothelial-like cells. Due to these characteristics, MSCs are good candidates for regenerative medicine and cell therapy strategies. The risk of transmitting viruses from ex vivo expanded MSCs is of particular concern, especially in immunosuppressed patients. Parvovirus B19 (B19V) is a human pathogenic virus with a specific tropism for bone marrow erythroid progenitor cells and the property to persist in several tissue types, including bone marrow. The virus can also cross the placenta and infect fetal erythroid progenitors. Thus, we investigated the susceptibility and permissiveness of bone marrow and placenta-derived MSCs to B19V to understand their potential role in the risk of B19V transmission. Mononuclear cells were isolated by Ficoll-Paque centrifugation of bone marrow blood from healthy donors and grown in DMEM containing 20% FBS at 37°C in 5% CO_2. Non-adherent cells were removed after one week and medium regularly changed. Expression of surface antigens, specific for defining the identity of MSCs, was measured by flow cytometry, as well B19V receptor. For infection experiments, cells were inoculated with B19V viremic serum in order to obtain a moi of 10^4 geq/cell. After the absorption period of 2 h at 37°C, infected cells were washed and seeded at an initial density of 10^6 cell/ml. Aliquots of cells were collected at different time post infection and analyzed for the presence of viral DNA and RNA by qPCR and qRT-PCR assays. Cells expressed the standard MSC markers, such as CD29, CD44, CD73, CD90, and OCT-4 and expression was maintained after infection. The B19V receptor globoside was expressed in most cells. Viral DNA became associated to cells, but analysis of viral nucleic acids revealed that neither replicative nor transcriptional activity occurred. However, viral DNA persisted in late time post infection. Our results showed that MSCs are susceptible but not a permissive environment to B19V productive infection and the presence of B19V DNA did not alter the expression profile of surface antigens. Further investigations are needed to understand the possible consequences on the cell physiology, like an impaired differentiation ability, as well the capability of virus reactivation after cell differentiation.

21 - Coinfection by Human Cytomegalovirus and Herpesvirus type 6 can trigger cell fibrosis: possible impact on scleroderma.

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Coinfection by Human Cytomegalovirus and Herpesvirus type 6 can trigger cell fibrosis: possible impact on scleroderma.

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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 characterized by vasculopathy and multi-organ fibrosis. Both viruses have a tropism for endothelial and fibroblast cells, deeply involved in SSc disease; *in vitro* infection with HCMV and HHV-6 induced the expression of pro-fibrotic factors in primary human dermal fibroblasts and modulated the expression of miRNAs recognized for their key function in fibrosis. Although these two viruses are often simultaneously reactivating in the adult host, where they can enhance each other, no data are available on their joint effect. Thus, we wanted to assess whether the co-infection by HCMV and HHV-6 could trigger cell fibrosis more than either virus alone. To this purpose, primary human dermal fibroblasts, were individually infected or co-infected with HCMV and HHV-6A, and the expression of miRNAs and cell factors correlated to fibrosis/apoptosis was investigated.

Materials and Methods: Human primary dermal fibroblasts were infected *in vitro* with cell-free inocula of HCMV and HHV-6A, and at different times post infection (0, 1, 2, 4, 7 and 10 d.p.i.) were collected to extract nucleic acids. The analysis was carried out by specific qPCR microarrays, detecting and quantifying 84 human microRNAs associated with cell fibrosis, and 84 factors associated with fibrosis or apoptosis.

Results: Concurrent HCMV/HHV-6A infection significantly modulated the expression of up to 58 miRNAs, 57 fibrosis-associated and 42 apoptosis-associated factors, evidencing significant differences between double-infected and single-infected cells and showing for the first time that the two viruses can cooperate in inducing alterations potentially leading to cell fibrosis.

Discussion and Conclusions: HCMV/HHV-6A infection profoundly remodulate miRNAs and transcriptional factors in human dermal fibroblasts. The correlation between these *in vitro* results with *in vivo* observations support a role of HCMV and HHV-6 in the multistep pathogenesis of fibrosis in SSc. Further work is required to definitely answer the question of whether beta-herpesviruses are causally linked to the disease and to enable the possible use of targeted antiviral treatments to improve clinical outcome.

27 - Prevalence analysis of urinary and plasma JCPyV, BKPyV, MCPyV, HPyV6, HPyV7 and QPyV in patients infected with HIV-1.

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Prevalence analysis of urinary and plasma JCPyV, BKPyV, MCPyV, HPyV6, HPyV7 and QPyV in patients infected with HIV-1.

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Introduction: a direct link between immunosuppression and the development of opportunistic infections has been established and it is also known that Human polyomaviruses (HPyVs), that commonly infects healthy humans, have a clearly established potential for causing severe organ damage or malignant transformation, especially in individuals with weakened immunity. For this reason, since it is well defined that HIV/AIDS predisposes to viral infection, persistence, or reactivation, in this study the HPyVs prevalence among HIV-1 infected patients and a possible correlation between HPyVs and HIV sero-status, were investigated.
Materials and Methods: qualitative PCR were performed to amplify the Viral Capsid protein 1 (VP1) and the Large T antigen (LT) of JCPyV, BKPyV, MCPyV, HPyV6, HPyV7 and QPyV. Samples tested positive to HPyVs DNA detection were subjected to a quantitative real time PCR (qPCR) in order to quantify the DNA viral amount. The Non-Coding Control Regions (NCCRs) of samples tested positive, were also amplified by nested PCR.
Results: Forty-four urine (44/78, 56%) and 16/78 (20,5%) plasma samples resulted positive for JCPyV, while 19 out of 78 urine (24%) and 4/78 (5%) plasma samples were positive for BKPyV. MCPyV was detected in 22 out of 78 urine (28%) and in 8 out of 78 plasma sample (10%); HPyV6, HPyV7 and QPyV, were not detected in any sample. qPCR showed a JCPyV load mean value of $3x10^7$ in urine and of $6x10^5$ in plasma. For BKPyV the viral load mean value was of $1x10^5$ in urine and of $2.5x10^3$ in plasma. Finally, the MCPyV load mean value was of $5x10^3$ in urine and of $1x10^3$ in plasma. The Non-Coding Control Region (NCCR) of HPyVs positive samples was amplified by nested PCR and sequenced. As expected, results showed an archetype NCCR structure organization in all analyzed urine samples and in MCPyV plasma samples. An archetype NCCR organization with the occurrence of some points mutations was observed in all JCPyV and BKPyV plasma samples.
The detection of HPyVs shown a significant correlation with a low level of CD4+, around 200 CD4+/mm^3, supporting a possible role of HIV in HPyVs infection. No significant association was found between the presence of HPyVs DNA and HPyVs viral loads versus age, gender and HIV-1 load at enrollment.
Discussion and Conclusions: further studies are warranted in order to define the clinical importance of JCPyV, BKPyV and MCPyV DNA detection in HIV-1 patients. HPyV6, HPyV7 and QPyV, instead, were not detected in any sample confirming, as previously described, that these HPyVs seems not to have a clinical relevance in these patients.

28 - CLINICAL AND PROGNOSTIC SIGNIFICANCE OF MERKEL CELL POLYOMAVIRUS (MCPyV) IN NON-SMALL CELL LUNG CANCER (NSCLC)

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CLINICAL AND PROGNOSTIC SIGNIFICANCE OF MERKEL CELL POLYOMAVIRUS (MCPyV) IN NON-SMALL CELL LUNG CANCER (NSCLC)

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Background: Merkel cell polyomavirus (MCPyV) is a double-stranded DNA virus associated with Merkel cell carcinoma (MCC). Considering the widespread prevalence of the virus across the body, the involvement of MCPyV in tumours other than MCC, such as non-small-cell lung carcinoma (NSCLC), cannot be excluded. A potential association between MCPyV infection and EGFR expression it has also been suggested since BRAF gene, a downstream target of EGFR pathway, was found to be higher expressed in MCPyV+ samples than negative ones. Moreover, this correlation, it would seem associated with worse prognosis. Therefore, in this study the prevalence of MCPyV in NSCLC, and the prognosis through the screening of EGFR were investigated.

Materials and Methods: Formalin-fixed paraffin-embedded tissue (FFPE) and corresponding nonmalignant lung tissue were obtained from 90 NSCLC patients. After DNA and RNA extraction, to detect MCPyV DNA, a quantitative real-time PCR (qPCR) was performed. To study the expression of viral RNA transcripts and virally encoded protein, a qualitative PCR, employing three set of primers (LT1, LT3 and VP1), was used. EGFR mutation analysis was carried out. PCR products were sequenced in service.

Results: MCPyV DNA was detected in 14/90 FFPE and in 1/90 nonmalignant lung tissue. qPCR showed viral DNA loads ranging from $1x10^2$ to $5.5x10^2$ copies/ug. By qualitative PCR, MCPyV DNA were detected in 3 samples with LT1 primer, in 7 with LT3 primer, and in 4 with VP1 primer. Four of ten samples also expressed the LT gene transcript, whereas no VP1 gene transcript was found. Sequence analysis of the LT gene of these 4 samples, showed in 3 samples, amino-acid substitutions at the C terminus of LT. None of these mutations caused stop codons. In contrast, the LT gene of 1 tumor, presented a frameshift mutation which generated stop codons. These mutations occurred downstream from the Rb-binding domain causing a truncated exon 2 encoding LT helicase. Analysis of EGFR showed that the infection rate of MCPyV, was higher in NSCLCs with EGFR mutations than without EGFR mutations; however, this difference was not statistically significant (>0.05).

Discussion and Conclusions: Although the viral prevalence in NSCLCs was low, the tumor-specific molecular signatures support the possibility that MCPyV could be associated with the pathogenesis of NSCLCs. Moreover, since MCPyV infection is observed in occurrence of EGFR mutation, our results could indicate that MCPyV could be considered an EGFR mutagen and might spark interests to deepen the prognostic value of EGFR in NSCLCs.

29 - Prevalence of Merkel cell polyomaviruses, human polyomaviruses 6, 7 and Trichodysplasia Spinulosa-associated polyomaviruses in Actinic Keratosis Biopsy Specimens

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Prevalence of Merkel cell polyomaviruses, human polyomaviruses 6, 7 and Trichodysplasia Spinulosa-associated polyomaviruses in Actinic Keratosis Biopsy Specimens

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Introduction: To date, 14 human polyomaviruses (HpyVs) have been identified using high-throughput technologies. Among them, Merkel cell polyomaviruses (MCPyV), human polyomaviruses (HpyV6, HpyV7) and Trichodysplasia Spinulosa-associated polyomaviruses (TSPyV) present a skin tropism, but a causal role in skin diseases has been established only for MCPyV as causative agent of Merkel cell carcinoma (MCC) and TSPyV as etiological agent of Trichodysplasia Spinulosa (TS). In this study, we investigated the prevalence of MCPyV, HPyV6, HPyV7 and TSPyV in actinic keratosis (AK).

Materials & Methods: Nine consecutive patients with clinical diagnosis of AK have been recruited in our center. A lesional and a non-lesional skin biopsy have been performed in each patient to confirm clinical diagnosis and to investigate the prevalence of MCPyV, HPyV6, HPyV7 and TSPyV in AK, a premalignant skin lesion that has the potential to progress towards a squamous cell carcinoma (SCC). Positive HPyVs DNA samples were subjected to qualitative PCR with different specific primer pairs mapping VP1 and LT regions of the genome and subsequently sequenced.

Results: Nine patients with a mean age of 78.56 years, all male, have been enrolled in this study. All the lesions were found on the scalp, the 78% of AK were grade I (according to Olsen 1991); 22% of the lesions was Bowenoid AK, while 78% was atrophic AK at histological analyze. The presence of MCPyV, HPyV6, HPyV7 and TSPyV DNA in lesion and non-lesion skin sections was determined by qualitative PCR. MCPyV DNA was analyzed using primers amplifying the VP1 region of the genome (307bp). MCPyV DNA was found in all lesion tissue samples (9/9; 100%), and in 6/8 (75%) non-lesion biopsy samples. This finding confirms that MCPyV is part of cutaneous microbiota and is commonly isolated from skin samples of adult subjects. A qualitative PCR analysis, using primers amplifying the LT region of the genome was performed to find HPyV6 and HPyV7. HPyV6 was found only in 1/8 (12.5%) healthy tissue samples, while HPyV7 was not found in any samples. In this study none of the biopsies tested were found positive for TSPyV. All biopsy samples found positive for MCPyV showed a wild type VP1 sequence. P1, found positive to HPyV6, showed a LT sequence with two-point mutation, compared to the reference one: the first is 73A>G and the second is 135A>G.

Discussion: These results seem to confirm that HPyV6 is, like MCPyV, part of the skin microbiota whereas, the absence of HPyV6 and HPyV7 in AK samples could confirm that these two HPyVs are not involved in cutaneous malignancies. Future research to establish a possible role of MCPyV, HPyV6, HPyV7, and TSPyV in AK should include investigation of the genome copy number, the state of the viral genome, and the expression of viral genes.

32 - Epidemiology of viral agents causing acute gastroenteritis in children in Northern Italy (2019-2021): focus on Norovirus circulation.

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Epidemiology of viral agents causing acute gastroenteritis in children in Northern Italy (2019-2021): focus on Norovirus circulation.

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Introduction. Acute gastroenteritis (AGE) with viral aetiology are a leading cause of mortality and morbidity, especially among children. There are nearly 1.7 billion cases of childhood diarrheal disease worldwide every year. Rapid diagnosis optimizes patient treatment and reduces hospitalization. This survey carried out in Parma (Northern Italy) from 2019 to 2021 aimed to assess the viral epidemiology of AGE in children using a multiplex polymerase-chain reaction (PCR) platform, and focusing on Norovirus (NoV) genogroup GI/GII dynamics.
Materials and Methods. In this study, 2,026 stool samples from as many children aged 0-14 years with AGE symptoms (vomiting, diarrhoea, abdominal pain, dehydration, and fever) were analysed with FilmArray Gastrointestinal Panel (BioFire Diagnostics) and Enterovirus-targeting PCR (BioMérieux). Conventional diagnostic assays (culture isolation and microscopic examination) were also carried out. Laboratory diagnosis was performed upon medical order.
Results. Overall, 1,386 potential enteric pathogens (bacteria, viruses and parasites) were detected in 1,001 (1,001/2,026, 49.4%) samples, as single or mixed infections. Of these, 515 viruses (515/1,386, 37.1%) were detected in 450 samples (450/2,026, 22.2%; 49.4% *vs* 22.2%, $P<0.0001$). NoV was most frequently detected (161/1,386, 11.6%), followed by Enterovirus (109/1,386, 7.9%) and Rotavirus (99/1,386, 7.1%). NoV single infections (92/161, 57.1%) prevailed on mixed infections (69/161, 42.9%). NoV prevalence was highest in reference to total viruses detected in each year (66/266, 24.8% in 2019, 35/94, 37.2% in 2020, and 60/155, 38.7% in 2021, respectively). The NoV rate decreased in 2020 when compared to 2019 and 2021 (- 46.9% *vs* 2019 and - 41.6% *vs* 2021). In 2021, NoV rate was similar to that of 2019 (60 *vs* 66 cases, respectively).Viral AGE mostly affected children aged 0-5 years (376/450, 83.5%), male (258/450, 57.3%) and inpatients (286/450, 63.5%). Similarly, NoV was more frequent in children aged 0-5 years (133/450, 29.5%), male (103/450, 22.8%) and inpatients (103/450, 22.8%). NoV showed seasonal prevalence in winter in 2019 and 2021, while it was not found from April to October 2020.
Discussion and Conclusions. A prompt molecular diagnosis improves the surveillance of the burden associated to AGE, increasing the sensitivity of pathogen detection. Using a multiplex PCR, we found a highest prevalence of NoV mainly in young inpatients, highlighting the occurrence of severe AGE requiring hospitalization. NoV rate decreased in 2020, probably as a consequence of the SARS-CoV-2 pandemic, imposing a strengthening of restriction measures and decreasing the number of samples received. This aspect deserves attention, since NoV may cause severe AGE and vaccines are still under study.

39 - Genomic sequence analysis of 8 SARS-CoV-2 strains circulating in Parma (Northern Italy) during the first and the second wave of COVID-19 pandemic.

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Genomic sequence analysis of 8 SARS-CoV-2 strains circulating in Parma (Northern Italy) during the first and the second wave of COVID-19 pandemic.

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Introduction. A growing number of emerging SARS-CoV-2 variants is being identified worldwide, impacting on COVID-19 severity, transmission, diagnostics, therapeutics, and natural and vaccine-induced immunity. This study reports the genomic sequences of 8 SARS-CoV-2 strains from patients with respiratory disease admitted to the University Hospital of Parma (Italy) during the first (February-May 2020) and the second (October 2020-March 2021) wave of the pandemic.

Materials and Methods. The detection of SARS-CoV-2 RNA in 8 samples (and in 4 cases also after cell culture detection) was carried out by rRT-PCR of the Centers for Disease Control and Prevention (CDC, Atlanta, USA) after RNA extraction by "EASYMAG®/EMAG®" (Biomerieux, France) or "Nimbus IVD" (Seegene Inc, Seoul, Korea). The amount of extracted RNA was checked by "Nanodrop" (Thermo Fisher Scientific, Inc) and 3 were sent to the company "Biogem S.c.a.r.l." (Ariano Irpino, Italy) and 5 to "IGA Technology Services" (Udine, Italy) for the sequence analysis.

Results. The most common mutation observed in 7/8 strains was the A-to-G mutation at position 23,403 of the Wuhan reference strains resulting in SARS-CoV-2 "D614G variant" that leads to the Spike protein amino acid change "D614G" (which alters infectivity and virulence and can increase the mortality). FASTA was available only for 5 strains: "PangoLEARN" software identified these strains as "SARS-CoV-2 variants" in comparison with the reference SARS-CoV-2 strain "MN908947.3" and the following lineages were assigned: 3 United Kingdom (variant α)-Lineage B.1.1.7, 1 Large European Lineage B.1.160 and 1 South Africa (variant β)-Lineage B.1.351. The genomic sequences of these strains were deposited in GISAID; the remaining 3 strains are under investigation for the lineage assignment.

Discussion and Conclusions. In the study, as expected with respect to the variants circulating in Europe during the study period, we found 3 "B.1.1.7-variant α United Kingdom" and one "B.1.351-variant β South Africa". Moreover, one strain detected in a nasal swab of a one-year-old Indian infant, belonged to lineage B1.160 named variant "Large European Lineage". This variant represents an EU/EEA and UK multi-country cluster circulating in Belgium, France, Germany, the United Kingdom, and Italy and has recently been shown to be responsible for the first evidence of transmission from human to dog. Phylogenetic analysis and homology modelling have added knowledge to the fine details of SARS-CoV-2, as well as the studies exploring the genome of the virus and the structure of its proteins. The continuous search for viral variants with decreased or increased pathogenic potential would be a significant step and it should be routinely performed.

48 - Study of Parvovirus B19 translocation across the placenta using the BeWo model

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Study of Parvovirus B19 translocation across the placenta using the BeWo model

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Introduction
Parvovirus B19 (B19V) is a human pathogenic virus characterized by a selective but not exclusive tropism for erythroid progenitor cells. In pregnancy, B19V poses a potential risk to the fetus as it can spread transplacentally leading to serious fetal complications such as anemia and hydrops, cardiomegaly and pericardial effusion, spontaneous abortions and intrauterine death. Although the etiologic role of B19V in these adverse outcomes has been widely recognized, the interaction between B19V and the human trophoblasts cells within the placenta has not yet been comprehensively explained.
Materials and Methods
We have therefore established *in vitro* experimental models based on the BeWo human choriocarcinoma cell line. BeWo cells show trophoblast-like appearance and form a confluent, polarized monolayer resembling first trimester placenta in structure and function; they can also be induced to undergo terminal differentiation and syncytialization, thus mimicking a mature placenta, when cultured in the presence of forskolin. A BeWo polarized monolayer using a collagen-coated membrane as scaffold was established, where the integrity was validated by cell morphology, biophysical features, and immunostaining of specific membrane proteins. Once obtained, these experimental models were used to detail B19V infection of human trophoblasts, and its transport across the placental barrier.
Results
The presence of the globoside as a cellular receptor was confirmed by cytofluorimetric analysis both on the cytotrophoblasts and on the syncytiotrophoblasts and evaluated at similar levels (25.7 ±10% and 28.1 ±9.8%, respectively). No evidence of productive infection was observed in the undifferentiated and forskolin-treated BeWo cells (50 µM) since viral DNA amounts gradually decreased in cells and in the corresponding supernatants. In addition, no mRNAs and accumulation of capsid proteins were detected in the unpolarized BeWo cell monolayers along a time course of infection, up to 72h. In polarized trophoblasts, a transcytosis mechanism can occur as demonstrated by kinetics of viral DNA accumulation.
Discussion and Conclusions
Knowledge on how B19V interacts with maternal-fetal interface will help to establish a suitable system to evaluate the effectiveness of the protective mechanisms operated by maternal antibodies against intrauterine infection, to assess the inhibitory activity of antiviral drugs and other therapeutic options that are not currently present.

53 - Transcriptional analysis of human bronchial epithelial cells with and without CFTR modification after SARS-CoV-2 infection

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Transcriptional analysis of human bronchial epithelial cells with and without CFTR modification after SARS-CoV-2 infection

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Introduction: Some evidence showed reduced spread of SARS-CoV-2 in people with cystic fibrosis, suggesting a complex regulation of SARS-CoV-2 replication. **Lotti et al demonstrated, by in vitro molecular analysis of SARS-CoV-2 infection in CFBE41o- cells, a higher viral load in WT cells compared to CFTR-modified one. Since the possible mechanism was not clarified, we** tried to shed light on this difference in SARS-CoV-2 infection, investigating the transcriptional landscape of the human bronchial epithelial cells with and without CFTR modification, before and after viral infection.
Methods: To reach our goals, we performed a global RNA sequencing of wild-type and CFTR-modified human bronchial epithelial cell lines before and after SARS-CoV-2 infection. We used Illumina library preparation protocols and run sequencing on an Illumina HiSeq2000 platform. The obtained data were firstly analysed to identify differentially expressed genes in different conditions (mutated vs wild type) and time points. To better understand the functional differences end effects of mutation or SARS-CoV-2 expression, we applied a Gene Set Enrichment Analysis (GSEA); the identified molecular signatures were explored in terms of functionally relevant sets of genes according to gene ontology and other specific categories.
Results: As first, we were able to find 716 differentially expressed genes analysing WT and CFTR-modified cells before SARS-CoV-2 infection, emphasising the different gene expression of cells related to CFTR mutation. At different time points after SARS-CoV-2 infection, in both WT and CFTR-modified cells were expressed, time dependently, different SARS-CoV-2 related genes. With the analysis of samples 96 hours after SARS-CoV-2 infection, by highlighting the different transcriptional profile present between WT and CFTR-modified cells, we were able to demonstrate that more than 800 genes, related to SARS-CoV-2 infection, were differentially expressed.
Discussion and Conclusions: We can therefore conclude that the presence of the CFTR mutation gives a distinct specific transcriptional phenotype compared to WT cells. Moreover, we demonstrate that both cell types were infected by SARS-CoV-2, even though its genes are expressed in a different, time-dependent manner. Therefore, it is possible to state the presence of distinct and different SARS-CoV-2-induced transcriptional profiles. Taken together these results, other than confirming the different behaviour of SARS-CoV-2 when infecting WT or CFTR-modified cells as we previously reported, provide crucial information on its molecular basis.

56 - Anti-HSV-1 Activity of natural raw and roasted unsalted pistachio kernels in human monocytic THP-1 and Vero cells.

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Anti-HSV-1 Activity of natural raw and roasted unsalted pistachio kernels in human monocytic THP-1 and Vero cells.

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Introduction. A great interest is globally devoted to the search for alternative treatment for HSV infections. To date, acyclovir and related nucleoside analogues are effective viral DNA polymerase inhibitors, though their intensive use has led to the increase of drug-resistance. Plants are rich sources of pharmacologically active agents, which provide several advantages such as reduced side effects, less resistance, low toxicity and various mechanisms of action. The purpose of the current study was to investigate the effects of natural raw (NRRE) and roasted unsalted (RURE) pistachio polyphenols-rich extracts in both human monocytic cells THP-1 and epithelial VERO cells on HSV-1 replication. **Materials and Methods.** Pistachio polyphenolic extracts were prepared following two different extraction methods, with or without *n*-hexane. Cell viability was measured by monitoring the metabolic activity over time following NRRE and RURE dose-dependent (0.2, 0.4, 0.8 mg/mL) treatment. Cells and virus dilutions were pre-treated with both extracts for 1h and mixed to allow viral adsorption. After 1 h, any unabsorbed virus was aspirated and the monolayer covered with Dulbecco's Modified Eagle's Medium containing 0.8% methylcellulose in presence of both extracts, separately. **Results and Conclusions.** The identification and quantification of phenolic compounds showed that the NRRE extracts were generally richer in polyphenolic compounds compared with RURE extracts. A better profile of cellular tolerability was reported for both NRRE and RURE extracts obtained without using *n*-hexane in both cell lines. Non-toxic concentrations of NRRE and/or RURE (0.6, 0.4 and 0.3 mg/mL) were employed to verify the antiviral effect by plaque reduction assay. In Vero cells, NRRE and RURE exhibited a significant inhibitory activity at 0.6 mg/mL and in particular, treatment with the mixture extracted with *n*-hexane determined a significant reduction of plaque numbers and size, as reported by change of morphology and by detection of micro plaques. Based on this, NRRE and RURE *n*-hexane were used to treat cells and virus suspension and detect the viral DNA and genes. We report that NRRE significantly reduced viral DNA and genes, whereas RURE mildly affected the viral DNA but partially reduced viral transcripts and protein at 0.6 mg/mL. A strong reduction was also detected in the intracellular virus production in THP-1 cells pre-treated with NRRE and RURE *n*-hexane and infected with HSV-1 by plaque assay. These data support the potential use of pistachio extracts as antiherpetic agents.

Acknowledgements: The work was funded by American Pistachio Growers (CA, USA).

58 - Broad range antiviral properties of small molecules against enveloped viruses

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Broad range antiviral properties of small molecules against enveloped viruses

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Introduction The global health emergency caused by the SARS-COV2 pandemic in the last 3 years has highlighted the need for broad-spectrum antiviral drugs capable of fighting new unknown viruses (panviral agents). Some panviral agents, for instance, degrades the envelope of coated viruses and are a promising weapon to prevent new pandemic waves. In this context, we have tested the antiviral effect of new oxidating small molecules(MAS). MAS, in silico designed and synthesized by University of Siena, oxidies the lipids present on the viral envelope and hinders viral entry .

Methods To evaluate their antiviral activity, these compounds were tested against the following three viruses: Herpes simplex type 2 (HSV-2), Chikungunya (CHIKV) and Coxsackie virus B5 (COX-B5). HSV-2 and CHIKV are both enveloped viruses but have different genomes, DNA and positive single strand RNA (+ssRNA), respectively. In contrast, COXB5 is a naked virus with +ssRNA genome and used as a negative control. Cytotoxicity assays were performed to establish the range of concentration without toxic effect on cell lines used: A549 and Huh-7. The antiviral activity was evaluated by limited dilution and plaque assays by incubating viruses directly with the compounds. Western blot (WB) and real time PCR were used to confirm reduction of viral proteins in infected cells and of genome production, respectively.

Results All compounds did not show citotoxicity up to 50 micromolar. MAS 584, 589 e 600 strongly inhibited replication of HSV-2 and CHIKV in A549 and Huh-7 cell lines, respectively. Viral proteins were also drastically reduced as demonstrated by WB. All compounds did not prevent infection by COX-B5, a nude virus.

Conclusion Selective degradation by oxidation of the viral membrane confers to MAS compounds the ability to prevent cell infections against enveloped viruses belonging to different families. It is possible to develop treatments based on these compounds that may prevent a wide range of infections. In all, MAS are promising panviral agents.

62 - The peptide A-3302-B isolated from a marine bacterium Micromonospora sp. inhibits HSV-2 infection by preventing the viral egress from host cells

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The peptide A-3302-B isolated from a marine bacterium Micromonospora sp. inhibits HSV-2 infection by preventing the viral egress from host cells

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Introduction. Despite antiviral drugs against herpetic infections, the increasing appearance of drug-resistant viral strains and their adverse effects prompt the research of novel antiherpetic drugs for treating lesions. Peptides obtained from natural sources have recently been considered a promising class of bioactive molecules for various biomedical applications including antiviral therapy. In this work, we investigated the antiviral activity of the peptide A-3302-B, isolated from a marine bacterium, Micromonospora sp. MAG 9-7, a rare actinomycete, against HSV-1, HSV-2 and HCMV.

Materials and Methods. From the purified crude extract of the strain MAG 9-7, isolated from marine sediment in Thailand, A-3302-B was identified by NMR analysis. Plaque reduction assays and virus yield reduction assays were performed to evaluate the antiviral activity in vitro. Its mechanism of action was explored by specific assays to evaluate the inhibited step of the viral replicative cycle by immunoblotting analysis, time of addition assays and transmission electron microscopy (TEM) analysis.

Results. A-3302-B exerted antiviral activity against HSV-2 with EC50 value of 14.22 microM whereas no inhibitory activity was observed against HSV-1 and HCMV. Furthermore, it was active also against an HSV-2 strain resistant to acyclovir previously generated in our laboratory. The peptide did not exert intrinsic virucidal activity and did not affect the expression of viral proteins
(ICP4, ICP8, and gD) at 4 and 16 hours post infection. Early cell membrane–virus interactions were not affected by the peptide. By contrast, A-3302-B affected later steps of the HSV-2 replicative cycle: it reduced the cell-to-cell virus spread and the transmission of the extracellular free virus by preventing the egress of HSV-2 progeny from the infected cells as demonstrated by TEM analysis.

Discussion and Conclusions. We demonstrated a specific anti-HSV-2 activity of A-3302-B. The peptide turned out to be a late inhibitor of HSV-2 infection, preventing the egress of newly produced viruses from host cells. This putative mechanism of action is supported by the following experimental evidence: (i) A-3302-B is active when administered after the viral inoculum; (ii) The expression of viral proteins and the assembly of infectious viral particles are preserved; (iii) The cell-to-cell spread of virions is inhibited in treated cellular monolayers; (iv) The release of extracellular free virus in culture supernatant is strongly reduced. The dual antiviral and the known reported anti-inflammatory activities of A-3302-B, and its effect against an acyclovir-resistant HSV-2 strain are attractive features for developing a drug to reduce the transmission of HSV-2 infections.

73 - Evaluation of the effect of time of addition of several compounds against SARS-CoV-2 and other RNA viruses

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Evaluation of the effect of time of addition of several compounds against SARS-CoV-2 and other RNA viruses

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Introduction

SARS-CoV-2 is a positive sense, single stranded RNA coronavirus that causes COVID-19, the respiratory illness responsible for the present pandemic. This pandemic highlighted lack of broad-spectrum drugs against infection by emerging viruses. Many laboratories began the search for drugs effective at blocking the onset of infection, or at blocking virus replication. In collaboration with the Pharmaceutical Chemistry group of the University of Sassari, a series of molecules of different chemical nature were selected. The antiviral activity of the molecules had previously been demonstrated on specific viruses. The aim of the study was the study of the mechanism of the antiviral activity of these compounds against SARS-CoV-2.

Materials and Methods

To evaluate the activity and the mechanism of action of the compounds against virus replication, HuH7 and VERO-TMPRSS2 cells were infected with 0.1 multiplicity of infection of SARS-CoV-2 in the presence of various concentrations of drugs. Time of addition experiments were carried out for the selected drugs to try to understand how they work and by what mechanism. Supernatants of infected cells were analyzed using qRT-PCR 48 hours post infection, while the cell lysates were analyzed by Western Blotting. These compounds were also tested against Zika Virus (ZIKV) and Vesicular Stomatitis Virus (VSV) using plaque assay on A549 cells.

Results

qRT-PCR showed that some of the drugs tested effectively inhibit SARS-CoV-2 infection, but this efficacy was only observed for a specific time of addition, different for each drug. This was also confirmed by Western Blots, which showed a reduced amount of Spike protein in infected and drug-treated cells. Plaque inhibition assays carried out by infecting cells with ZIKV showed, for some drugs, the same infection-reducing effect displayed on SARS-CoV-2, while for other compounds it detected no antiviral effect. As regards VSV, none of the compounds we tested had significant antiviral activity against it.

Discussion and conclusion

Given the encouraging results obtained, we believe that these drugs may be considered good candidates for the treatment of COVID-19. To this purpose, further studies will be needed to determine the mechanism of action of the compounds found to be most effective in inhibiting SARS-CoV-2 infection.

75 - Evaluation of neutralizing antibodies against Sars-Cov-2 in patients with Inflammatory bowel disease

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Evaluation of neutralizing antibodies against Sars-Cov-2 in patients with Inflammatory bowel disease

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to evolve worldwide generating new variants that are of concern because of their potential altered transmissibility and pathogenicity. The aim of this study was to evaluate the protective humoral response after administration of the second dose of vaccine (Pfizer-BioNTech mRNA BNT162b2) in patients with Inflammatory bowel disease (IBD). The results were also compared to a control group of naturally infected and uninfected vaccinated subjects in order to determine the production of vaccine-induced and naturally produced neutralizing antibodies (NtAbs) against the SARS-CoV-2 variants circulating in Italy.

Materials and methods: A total of 116 patients with IBD were recruited and divided into three subgroups according to the drug therapy administered (non-biological anti-TNFα (TNB), biological anti-TNFα (TB), and conventional therapy (TP)). The control group included 39 subjects with SARS-CoV-2 (COVID-19+) and 63 vaccinated subjects with two-dose of BNT16262. A single serum sample from each subject in the IBD and COVID-19+ groups, collected at 35-52 days after the positive swab, was analyzed by *in vitro* live virus-neutralization test. While for the uninfected subjects, blood samples were taken at one (V1) and three months (V3) after administration of the second dose of vaccine.

Results: In IBD patients, the highest Nt Ab titers were found for the Gamma variant (5.88% TNB; 11.11% TB; 10.90% TC) while the lowest were observed against the Omicron variant (47.05% TNB; 59.25% TB; 41.81% TC). On the other hand, significantly higher Nt Ab titers were found against B.1 and Alpha in COVID-19+ and vaccines, while lower were found against Delta, Gamma and Omicron variants.

Discussion and Conclusions: Results obtained and compared with the control groups showed that, despite being subjected to immunosuppressive therapies, IBD patients after vaccination showed a protective antibody titer against SARS-CoV2 variants, except for Omicron. In conclusion, this study shows that the vaccine is effective even in subjects considered frail.

76 - CHARACTERIZATION OF THE ANTI-RHINOVIRUS ACTIVITY OF 25-HYDROXYCHOLESTEROL AND 27-HYDROXYCHOLESTEROL, AND VALIDATION ON NASAL AND BRONCHIAL HISTOCULTURES.

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CHARACTERIZATION OF THE ANTI-RHINOVIRUS ACTIVITY OF 25-HYDROXYCHOLESTEROL AND 27-HYDROXYCHOLESTEROL, AND VALIDATION ON NASAL AND BRONCHIAL HISTOCULTURES

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Introduction: Human rhinovirus (HRV) is a quasispecies, a highly antigenically diverse virus population endowed with a high rate of mutations. The aim of this study is to provide an empirical proof of principle of the actual greater genetic barrier of 25-hydroxycholesterol (25OHC) and 27-hydroxycholesterol (27OHC), two physiologic oxysterols and host-targeting antivirals, using HRV as a quasispecies model. Moreover, we selected 27OHC for further studies aiming at exploring further its putative potential of preclinical development.

Materials and Methods: We first tested the antiviral efficacy of 25OHC and 27OHC by focus reduction assay. The toxicity profile of both oxysterols, along with their 50% cytotoxic concentration (CC50), was assessed by MTS assays and LDH assays. The ability of 25OHC or 27OHC to generate resistant strains of HRV was explored by exploiting clonal or serial passages approaches, and compared with the one of pleconaril and rupintrivir. Moreover, both the efficacy and biocompatibility of 27OHC were further validated in a challenging and predictive model, i.e. 3D in vitro fully reconstituted human nasal and bronchial epithelia from cystic fibrosis patients.

Results: 25OHC and 27OHC can block the infectivity of two different HRV strains, belonging to group A and B, at 50% effective concentrations (EC_{50}) in the low micromolar range, and are characterized by selectivity indexes ($SIs=CC_{50}/EC_{50}$) above 100. Moreover, we demonstrate with two different approaches that 25OHC and 27OHC do not select HRV oxysterol-resistant variants. Moreover, we demonstrate the ability of 27OHC to inhibit HRV yield in both nasal and bronchial epithelia, preventing virus-induced cilia damage.

Discussion and Conclusions: The complex of these characteristics suggests that 27OHC antiviral potential should be considered further, and provide a rationale for further studies aiming at exploring its potential of preclinical development.

81 - Characterization of Human Polyomavirus JC (JCPyV) infection in Human Neuroblastoma SH-SY5Y cells and evaluation of redox state during viral replication.

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Characterization of Human Polyomavirus JC (JCPyV) infection in Human Neuroblastoma SH-SY5Y cells and evaluation of redox state during viral replication.

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Background: Several intracellular factors, including the redox state, might affect the progression and outcome of viral infection. In physiological conditions, the redox balance is maintained by enzymatic and non-enzymatic systems, and it finely regulates several cell functions.While the role of some viruses in break this equilibrium and induce oxidative stress is well characterized, the role of redox state, during Human Polyomavirus JC (JCPyV) infection, is still poorly understood. JCPyV, a non-enveloped virus with a circular dsDNA genome, causes a lifelong asymptomatic and persistent infection in the reno-urinary tract of the adult population worldwide. In immunocompromised individuals, JCPyV can spread to the central nervous system (CNS) and cause a fatal demyelinating disease known as Progressive Multifocal Leukoencephalopathy (PML).

Understanding the mechanisms of the intracellular redox alteration, which occurs during viral infections and whether they could be associated with the progression of JC virus-induced diseases is of particular interest. Hence, the aim of this study was to unveiling role of redox state during JCPyV replication that govern the survival and death of cells infected by JCPyV.

Materials and Methods: SH-SY5Y cells were infected with the JCPyV archetype strain and collected 24hours post-infection. Viral DNA was extracted andquantified by quantitative RealTime PCR (Q-PCR). The expression of Viral Capsid protein 1 (VP1) and the activity of NADPH oxidase 2 and 4 (NOX2 andNOX4) were studied by Western blot (WB). The intracellularconcentration of glutathione (GSH)was assayed upon formation of S-carboxymethyl derivatives of free thiol with iodoacetic acid, followed by the conversion of free amino groups to 2,4-dinitrophenyl derivatives by reaction with 1-fluoro-2,4-dinitrobenzene.ROS were detected by cytofluorimetric analysis.

Results:JCPyV efficiently infects and replicates in SH-SY5Y cells. A loss in intracellular level of GSH and a ROS increase were observed. ROS increase depends mainly on NOX4, which is upregulated at both mRNA and protein levels. On the contrary, the expression of NOX2, the primary source of ROS in inflammatory cells, is downregulated.

Discussion and Conclusions:These data show redox alteration during JCPyVinfection and suggest that redox-sensitive pathways could represent novel cell-based targets for therapies aimed at blocking both viral replication and virus-induced persistence.

92 - B cell response six months after SARS-CoV-2 mRNA vaccination in people living with HIV under antiretroviral therapy

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B cell response six months after SARS-CoV-2 mRNA vaccination in people living with HIV under antiretroviral therapy

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Introduction. SARS-CoV-2 mRNA vaccines have demonstrated high immunogenicity in healthy subjects and preliminary results for people living with HIV (PLWHIV) are promising too. We have previously reported the persistence of spike-specific circulating IgG and memory B cells in healthy adults up to six months after mRNA SARS-CoV-2 vaccination. Unfortunately, limited longitudinal data are available for PLWHIV and no evidence of persistent spike-specific memory B cells have been reported yet. Whether the HIV chronic immune impairment affects spike-specific memory B cells remains a burning question.

Materials and Methods. We investigated the humoral response and the persistence of spike-specific memory B cells up to six months after vaccination with two doses of mRNA vaccines in 84 PLWHIV under antiretroviral therapy (ART) and compared them to healthy controls (HCs). Humoral response was analyzed with enzyme-linked immunosorbent assay and with a surrogate neutralization assay measuring angiotensin-converting enzyme 2 (ACE2) and receptor binding domain (RBD) binding inhibition. Spike-specific B cells were identified as S+/RBD+ and characterized for IgD and CD27 expression with a cytofluorimetric approach.

Results. Spike-specific IgG titers peaked 1 month after second dose and persisted up to six months after vaccination with no significant differences compared to HCs. The stratification of patients according to CD4+ T cell count showed a significantly lower IgG response in case of CD4<350/µl, remarking the relevance of immune reconstitution. The ACE2/RBD inhibition activity was detected in 58.4% of PLWHIV, compared to 86.2% in HCs. The amount of circulating spike-specific memory B cells detected in PLWHIV six months after vaccination was not significantly different from HCs, however there was prevalence of antigen-specific double negative (IgD-/CD27-) cells that are associated to precocious immune aging.

Discussion and Conclusions. In summary, our study provides real-world data on immunogenicity of SARS-CoV-2 mRNA vaccines in PLWHIV up to 6 months after vaccination. These results corroborate the opinion that PLWHIV with an adequate CD4+ T cells population (>350 cells/µl) and under ART can mount a significant humoral immune response after vaccination. Nevertheless, hints of altered immune responsiveness have been observed with ACE2/RBD binding inhibition assay and by spike-specific B cell phenotyping.

100 - Indomethacin inhibits human seasonal coronavirus replication at post-entry level

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Indomethacin inhibits human seasonal coronavirus replication at post-entry level

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Introduction: The non-steroidal anti-inflammatory drug (NSAID) indomethacin (INDO), a traditional cyclooxygenase-1 (COX-1) and -2 (COX-2) inhibitor with anti-inflammatory and analgesic properties, is known to possess antiviral activity against several viral pathogens, including the SARS-CoV coronavirus. INDO has been recently used successfully in the treatment of COVID-19 patients; however, very little is known on the mechanism of the antiviral activity. Herein we investigated the effect of INDO on human seasonal coronavirus (sHCoV) replication, using the alpha-coronavirus HCoV-229E and beta-coronavirus HCoV-OC43 as models. ***Materials and methods:*** Human lung MRC-5 fibroblasts were infected with HCoV-229E or HCoV-OC43 (0.1 $TCID_{50}$/cell) for 1h at 33°C. INDO, dissolved in ethanol, was diluted in culture medium at the time of use. Virus yield was determined by $TCID_{50}$ infectivity assay, and cell viability was determined by MTT assay. Viral and cellular proteins were evaluated by Western-blot analysis and HCoV genomic RNA (gRNA) levels were determined by qRT-PCR. For RNA transfection experiments, MRC-5 cells were transfected with HCoV-229E or HCoV-OC43 gRNA (1 µg/ml) at 33°C, and treated with INDO after transfection. ***Results:*** Indomethacin was found to have a remarkable cytoprotective and antiviral activity in both models, causing a dose-dependent decrease of infectious virus yield and extracellular HCoV gRNA levels. Treatment with INDO at different stages of the virus infection cycle resulted in significant virus titer reduction when treatment was started after virus adsorption (0-6h p.i.), whereas treatment of cells before infection or during the adsorption period did not affect virus replication. INDO was also effective in inhibiting virus replication in HCoV-229E or HCoV-OC43 gRNA transfection experiments, confirming that the NSAID does not interfere with virus entry. Analysis of viral proteins and RNA shows that INDO reduces HCoV nucleocapsid N and spike S protein levels, as well as intracellular viral RNA expression, an effect associated with drug-induced phosphorylation of the translation initiation factor eIF2α at Ser51. ***Discussion and Conclusions:*** Seasonal HCoVs generally cause mild upper respiratory tract diseases, although they may sometimes cause severe infections. Presently there is no specific treatment for sHCoV infections. The results described herein indicate that indomethacin is effective against HCoV-229E and HCoV-OC43 infection *in vitro*, acting at post-entry level and causing a block of viral RNA and protein expression in infected cells. These results suggest a possible beneficial effect of indomethacin in the treatment of human coronavirus infections.

107 - Infection rate of respiratory viruses in the pandemic SARS-CoV-2 period considering symptomatic patients: comparison between 2020 and 2021.

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Infection rate of respiratory viruses in the pandemic SARS-CoV-2 period considering symptomatic patients: comparison between 2020 and 2021.

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Background: In last two years, the SARS-CoV-2 pandemic has determined radical changes in human behaviours and lifestyles, with a drastic reduction of socialization due to physical distancing and self-isolation (https://www.governo.it/it/coronavirus-misure-del-governo). These changes have also been reflected in the epidemiological patterns of common respiratory viruses. For this reason, early discrimination of respiratory viruses is important as new variants emerge. **Methods:** Nasopharyngeal swabs of 2554 patients, with clinically suspected Acute Respiratory Infections (ARIs) from October 2019 to November 2021, were collected to detect one or more of the 23 common respiratory pathogens, especially viruses, via BioFilmArray RP2.1*plus*, including SARS-CoV-2. Demographical characteristics and epidemiological analyses were performed as well as laboratory features profile of positive patients. **Results:** An observational study on 2300 patients (254 patients were excluded because of missing data) including 1560 men and 760 women, median age of 64,5 years, has been carried out. Considering the respiratory virus research request, most of the patients were admitted at the Emergency Medicine Department (41.2%, of patients), whereas 29.5% were admitted at the Infectious Diseases Department. The most frequently detected pathogens included SARS-CoV-2 (31.06%, 707/2300, from March 2020 to November 2021), InfA-B (1.86%, 43/2300). The significative decrease of positive rate of SARS-CoV-2 was associated with the massive vaccination, especially in eldest people. **Conclusion:** This study represents a dynamic picture of the epidemiological curve of common respiratory viruses during the two years of pandemic, with a disregarded trend for additional viruses. The possible causes were attributable either to the use of masks, social distancing, smart working, and closure of schools, as measures implemented by the national policy to contain the spread of SARS-CoV-2 has further influenced the changes in the positivity rates of most respiratory viruses.vaccination campaigns and emerging of new SARS-Cov-2 variants.

110 - ARE III-GENERATION ANTIGENIC TEST SENSITIVE AS MOLECULAR TEST FOR DETECTION OF SARS-CoV-2?

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ARE III-GENERATION ANTIGENIC TEST SENSITIVE AS MOLECULAR TEST FOR DETECTION OF SARS-CoV-2?

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Background The pandemic due to SARS-CoV-2 infection has brought about enormous changes from every point of view, from social habits to certainties in the scientific world. The need to manage thousands of users daily and the need to quickly diagnose the presence of infection in patients for the containment of the spread, has led to the development of many different assays and methodologies, to couple to the gold standard PCR, reliable, but complex and long. Among these technologies are third generation antigenic assay, based on principle of microfluidric. **Methods:** The methods used and compared with each other were the Microlab Nimbus/Starlet technologies with Kit Allplex and LumiraDX Platform respectively for molecular and antigenic testing on nasopharyngeal swabs as starting material. The results of patients who had carried out both tests were analyzed and through the Cohen index the sensitivity of the antigen test compared to the molecular test was evaluated. **Results:** A total of 5040 patients were enrolled in this study in the period September-December 2021. All patients performed a nasopharyngeal swab for the search and identification of SARS-CoV-2 at the Emergency Unit of the Tor Vergata Policlinic, Rome. Of these 1603 patients underwent both tests analysed in this study showing a remarkable concordance in response (98.23%), but Cohen's index lowers this concordance to 64%. Only in 1.77% of cases the test result was discordant. **Conclusion**: The use of the gold standard PCR method represents the safety for the detection of nucleic acids specific to SARS-CoV-2. But the need for fast diagnosis and at low costs, makes the third generation technology, in the field of antigen tests, LumiraDX Platform an excellent system for referring patients also taking into account the symptoms and clinical history of the patient.

111 - Wastewater-based surveillance for the monitoring of SARS-CoV-2 circulation in Sicily

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Wastewater-based surveillance for the monitoring of SARS-CoV-2 circulation in Sicily

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Introduction: Wastewater-based epidemiology has been recognized as a complement for clinical disease surveillance. Several studies had demonstrated the advantages of environmental surveillance through wastewater monitoring for the assessment of viral circulation in a given territory. To this aim, the National Surveillance Network SARI (Sorveglianza Acque Reflue in Italia) group was established by the Istituto Superiore di Sanità (ISS) to monitor the presence of the SARS-CoV-2 genome in wastewater as a predictive tool for the prevalence of COVID-19 in the population. The Regional Reference Laboratory (RRL) of the PROMISE Department of the University of Palermo was included in the SARI network since June 2020. As a result of revisions to the list of sampling sites, from October 2021, receiving once or twice a week wastewater samples for virological analysis from 5 municipal wastewater treatment plants in Sicily: Modica (RG), Vittoria (RG), Ragusa (RG), Gela (CL) and Messina (ME).
Materials and Methods: In this study, 150 wastewater samples collected from October 1 2021 to June 30 2022 for virological investigations according to standard protocols approved by ISS (SARS-CoV-2 Surveillance Protocol in urban wastewater, SARI - rev. 3). Nucleic acids extraction from 4 ml of concentrated wastewater sample was optimized using MagNa Pure automatic extractor (Roche), and quantitative determination of SARS-CoV-2 genome was evaluated by RT-qPCR assays with specific primer sets. To assess the concentration/extraction/amplification efficiency of the method a process control virus solution (Murine Norovirus; MNoV) was added to each wastewater sample prior to concentration.
Results: Out of 150 samples collected from October 1 2021 to June 30 2022, 141 (94%) were positive for SARS-CoV-2, five negative (3.3%), while two showed an insufficient yield (<10%) and two were invalid. RNA quantization (genomic copies/µL) for all positive samples ranged between 1.75E+02 and 1.25E+06. Process yields calculated based on MNoV, ranged between 10.5% and 100%. Trend of SARS-CoV-2 concentrations in wastewater showed a significant increase (>30%) in SARS-CoV-2 genome concentrations in mid-June 2022, compared to the previous months.
Discussion and Conclusions: The results of this study confirmed the utility of wastewater surveillance as an important instrument to evaluate SARS-CoV-2 circulation into human populations.

The observed significant increase of SARS-CoV-2 loads suggested a rise in the number of individuals excreting SARS-CoV-2, which is in agreement with the rise of COVID-19 cases, possibly linked to the spread of the Omicron sublineages BA.4, BA.5 and BA.2.12.1.

116 - Emergence of novel Rotavirus G1P[8] strains in Sicily in 2021

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Emergence of novel Rotavirus G1P[8] strains in Sicily in 2021

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Introduction: Rotavirus (RVA) is a major etiologic cause of gastroenteritis in children. RVA are classified into G and P types on the basis of antigenic and genetic diversity of the capsid proteins VP7 and VP4, which elicit for neutralizing antibodies. Accumulation of point mutations, interspecies transmission and/or reassortment events result in the evolution of RVA. Recently, novel G1P[8] RVAs, phylogenetically different from the previously known lineages have emerged and spread in our territory. These novel strains are also distant from the strain of the monovalent Rotarix vaccine, recommended and offered in Sicily. This study describes the molecular investigations of G1P[8] strains detected in Palermo in 2021, aiming at better understanding their genetic evolution.
Materials and Methods: As part of the surveillance of viral gastroenteritis, a total of 268 stool samples, collected from children admitted to the Children's Hospital "G. Di Cristina" in Palermo, were analyzed in 2021. All RVA-positive strains were G/P typed by hemi-nested PCR and 21 G1P[8] strains were further analyzed by phylogenetic analysis of VP7 and VP4 regions, according to the genotyping recommendations of the Rotavirus Classification Working Group.
Results: In 2021, RVA accounted for 8.9% of gastroenteric infections, and G1P[8] genotype was detected in 87.5% of RVA-positive samples. The phylogenetic study conducted showed the presence of a novel cluster for both VP7 and VP4, clustered with a high percentage of nucleotide identity (>98%) and different from strains analyzed in previous years or vaccine-derived strains. Comparison of VP7 and VP4 sequences with Rotarix vaccine showed the presence of multiple nucleotide substitutions.
Discussion and conclusions: The local distribution of RVAs is affected by introduction of Rotavirus vaccine, leading to a radical reduction in the circulation of G1P[8] RVAs and hospitalizations for infections with it. Recently, emerging novel G1P[8] RVAs were introduced in Palermo, contributing to the seasonal epidemic in 2021. These new G1P[8] strains represent further evidence of the evolutionary capacity of RVAs. Molecular surveillance of circulating RVAs is important for the early detection of new mutants that might emerge under vaccine pressure and for the monitoring of the effectiveness of vaccine programs.

127 - PREVALENCE AND LOAD OF THE RECENTLY DISCOVERED REDONDOVIRUS IN SALIVA SAMPLES FROM HEALTHY AND COVID-19 SUBJECTS

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Prevalence and load of the recently discovered redondovirus in saliva samples from healthy and covid-19 subjects

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Introduction: Redondoviridae is a recently described family of viruses that appear restricted to the human oral and respiratory tract and have a prevalence of up to 15%. Redondovirus (ReDoVs) levels are higher in the airway of intubated patients and oral samples of periodontitis patients, suggesting that they could be used as a marker to define the severity of the pathological conditions. Several published studies suggest that ReDoV is not part of the normal oral and/or respiratory microflora of humans, differently from other circular single-stranded DNA viruses and that ReDoV infection might be involved in clinically relevant disorders.

Materials and Methods: A total of 358 saliva samples were investigated for ReDoV presence and loads by in-house developed PCR methods. 283 samples were from healthy individuals, and 99 from hospitalized SARS-COV-2 positive patients The median age in healthy subjects was 51 years, with 180 (63,6%) males. The hospitalized patients had 56 years in the median, 32 (32,3%) were males. Concomitantly to ReDoV, the presence and loads of ubiquitous TTV were also evaluated.

Results: Overall, ReDoV was detected in 213 of 358 (59%) saliva samples. Of 283 samples from healthy individuals, 157 (55%) were positive for ReDoV at a medium viral load of 3,6 log copies of ml of saliva. 56 of 99 (57%) saliva samples from hospitalized SARS-COV-2 positive patients whose 56 (56,6%) were RedoV positive with a medium load of 2,8 log copies/ml. TTV was found with a higher prevalence (65 and 77% in healthy and diseased subjects, respectively) and levels (3.7 and 3.6 log TTV copies/ml in healthy and diseased subjects, respectively).

Discussion and Conclusions: The study reveals that the novel ReDoV is prevalent in saliva samples of healthy and diseased individuals with DNA levels that are comparable with those measured for TTV. Further study will be needed for investigating the role of ReDoV in the respiratory tract and if its presence can be correlated to the severity of acute and chronic respiratory pathologies.

128 - Prolonged viral shedding and monkeypox virus isolation from seminal fluid

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Prolonged viral shedding and monkeypox virus isolation from seminal fluid

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Background: Monkeypox is a rare viral zoonotic disease that is transmitted from one person to another by close contact with infectious materials or large respiratory droplets. Since May 2022, a multi-country outbreak of monkeypox virus (MPXV) is ongoing, with increasing cases identified all across the world. The virus transmission properties may be one of the key aspects associated with this unprecedented event to be elucidated. **Methods**: We investigated the viral shedding in the first available semen sample collected after the diagnosis from 11 MPXV-positive patients diagnosed between 17 May and 12 June 2022, at the National Institute for Infectious Diseases "L. Spallanzani", Italy. MPXV-specific PCR assay targeting the viral tumor necrosis factor receptor gene was performed to evaluate viral DNA levels based on quantification cycles (Cq) values. For one of the patients, longitudinal semen and plasma samples collected from day 5 to day 19 from symptoms onset (fso) were investigated to monitor the persistence of the viral DNA shedding. In this case, virus isolation on Vero E6 cells was also performed in the first available semen sample to evaluate presence of infectious virus. **Results:** MPXV DNA was detected in semen samples collected after the diagnosis (mean time: 2 days, interquartile range (IQR): 1-2.5, range: 1-6) in 9 of 11 patients (82%) at Cq levels ranging from 22.7 to 41.2 (IQR: 25.1-30.8), with a mean value of 24.9. Monitoring the shedding in longitudinal samples, we showed that MPXV DNA was detected in the plasma sample collected on day 8 fso (Cq: 34.5), while the following samples resulted negative. MPXV DNA was detected in all semen samples tested during the period of observation, with Cq values ranging from 27.8 to 40.6. More importantly, live and replication-competent virus was isolated from the semen sample collected on day 6 fso (Cq: 29.3). **Discussion and Conclusions:** Our findings support the evidence that a prolonged shedding of MPXV can occur in the semen samples of infected patients for weeks after the onset of the symptoms (at least up to 19 days). But, more importantly, our results demonstrate that semen may contain a replication-competent virus and represent a potential source of infection. Further studies will establish whether infectious MPXV found in semen derives from local genital lesions, is associated with seminal cells, or if viral replication occurs in the genital tract. The understanding of the viral tropism for the genital tract and the origin of viral particles in the seminal fluid of infected men will shed light on the role of sexual transmission in the spread of MPXV infection.

132 - Development of SARS-CoV-2 Mpro inhibitors retaining in vitro activity against circulating SARS-CoV-2 variants of concern

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Development of SARS-CoV-2 Mpro inhibitors retaining in vitro activity against circulating SARS-CoV-2 variants of concern.

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Introduction. The peculiar cleavage recognition site combined with its high degree of conservation makes the main protease (Mpro) of SARS-CoV-2 an attractive therapeutic target. The aim of this work was to evaluate in vitro the anti-SARS-CoV-2 activity of a library of newly synthesized compounds designed against Mpro.

Materials and Methods. Peptidomimetic Mpro candidate inhibitors were rationally designed based on the available structural information and applying molecular docking and dynamics techniques. The synthesized peptidomimetic compounds were tested in an enzyme inhibition assay in vitro. A set of 10 hit compounds was tested in a cell-based assay using live virus to determine their ability to halt virus replication. VERO E6 cells were treated with non-toxic doses of each compound with or without the addition of 0.5 µM P-gp inhibitor (CP-100356) and subsequently challenged with the SARS-CoV-2 delta and omicron BA.2 variant at 0.001 MOI. Each experiment was performed at least in 2 independent runs and included a mock control, a virus back titration and the licensed Mpro inhibitor nirmatrelvir (NRM) as a reference compound. The inhibitory activity of each compound was determined by measuring the nucleocapsid expression by immunodetection and expressed as half-maximal inhibitory concentration (IC_{50}) using a non-linear fit normalization curve (GraphPad version 6.01).

Results. Among 46 compounds tested in vitro by enzymatic assay, four compounds were active in the sub-micromolar range (0.5 ± 0,2 µM) against Mpro enzyme. Compounds displaying enzyme inhibitory activities lower than 10 mM IC_{50} were further progressed to the cell-based antiviral assay. The CC_{50} of the selected compounds was >100 µM without CP-100356 but it decreased 4.4±1.7 fold for 3 compounds in the presence of CP-100356. In the absence of CP-100356, none of the compounds was able to inhibit virus replication while in the presence of CP-100356, 3 compounds (NF3101, NF3156, NF3021) were active against the delta variant (ID_{50} 1.6±0.5, 1.8±0.1, and 8.0±1.4 µM respectively) and against the omicron variant ((ID_{50} 5,8 ± 0,6, 2.5±0.2 and 9.5±0.5 µM respectively). A slight reduction in activity against the omicron variant (3.6-fold) was observed only for compound NF3101.

Discussion and Conclusions. We discovered novel SARS-CoV-2 Mpro candidate inhibitors inhibiting both delta and omicron variants of concern. The dependence by CP-100356 administration in VERO E6 cells was similar to those observed for the reference licensed drug NRM. The antiviral activity of the novel compounds currently remains around 50-fold lower than NRM but their less complex molecular scaffold will allow further structure-based optimization.

134 - In vitro evaluation of the virucide activity of nanostructured antimicrobial coatings for personal protective equipments

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In vitro evaluation of the virucide activity of nanostructured antimicrobial coatings for personal protective equipments

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INTRODUCTION

One of the most effective personal equipments in preventing infection by respiratory viruses is wearing surgical masks for mouth and nose protection. The aim of this study is to evaluate *in vitro* whether different nanostructured coatings applied to surgical masks have a virucide activity.

MATERIALS AND METHODS

The tested coatings contained the following chemical principles: zeine solution (a vegetal protein) in

ethanol/H_2O and in ethanol/lactic acid, aqueous solutions of $CuCl_2$ and NaOH (precursor to obtain nanometric cupper oxide), aqueous solution of $Zn(NO_3)_2$ and NaOH (precursor to obtain nanometric zinc), all at the same concentration (25 g/L). The coatings were obtained by soaking small coupons of TNT fabric in each solution for 30' followed by air drying. For cupper and zinc based coatings, the samples were soaked in a NaOH solution and oxidated at 60°C for 24h.

The virucide activity was studied against HCov-OC43, as a SARS-CoV-2 surrogate, Herpes Simplex Virus type-1, Human Adenovirus type 5 and Monkeypox Virus. The coupons coated with the different solutions were contaminated with the viruses under study and after 2' the fabric samples were soaked in 1ml of cell growth medium and vortexed for 1' to eluate the survived virus. Viral loads of these suspensions were titrated by end point dilution on cell cultures.

RESULTS

Preliminary experiments on surgical masks demonstrate a remarkable virucide activity of some coatings, though with different extent depending on the virus tested: the most effective ones appear to be zeine with lactic acid and the two cupper compounds (reduction $\geq$ 2 Log).

CONCLUSIONS

Some of these coatings applied to polypropylene surfaces were already tested and demonstrated a considerable virus inhibition (in some cases up to 3 Logs). Therefore, this strategy for virus transmission containment seems to be promising, providing some advantages in comparison with traditional systems, such as lower toxicity and/or ecocompatibility.

139 - Anti-SARS-CoV-2 Neutralizing activity of human breast milk from COVID-19 positive and negative lactating mothers

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Anti-SARS-CoV-2 Neutralizing activity of human breast milk from COVID-19 positive and negative lactating mothers

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Introduction: Immunity transfer through human milk is a key element in the infant's developing immunity. Recent studies have demonstrated the presence of antibodies against SARS-CoV-2 in human breast milk (HBM). Furthermore, it is known that aspecific antiviral activity not related to the presence of antibodies, is reasonably present in the HBM of all lactating women. The aim of the study was to detect the presence and magnitude of the neutralizing activity against SARS-CoV-2 of HBM from infected and previously infected lactating mothers (group A and B) and from mothers who never experienced SARS-CoV-2 infection (group C).
Materials and Methods: Forty-two women were enrolled, 11 in group A, 5 in group B and 26 in group C. Milk samples were collected 2 days (T0 - colostrum), 7 days (T1 - transition milk), 20 days (T2 - mature milk) and 30 days (T3) after the delivery. The SARS-CoV-2 50% neutralizing endpoint (NT_{50}) titers were determined at each time point, based on a microneutralization assay results. A semiquantitative enzyme-linked immunosorbent assay (ELISA) was performed to investigate the presence of anti-SARS-CoV-2 neutralizing antibodies.
Results: Milk samples from group A showed antiviral activity with median NT_{50} titers of 1:32.0 (T0, range 1:16.0 - 1:512.0), 1:55.2 (T1, range 1:19.8 - 1:90.5), 1:2.6 (T2). No neutralizing activity was observed at T3. Median NT_{50} titers in group B were 1:15.7 (T1, range 1:4.0 – 1:256.0) and 1:6.7 (T2, range 1:2.3 – 1:20.7). Milk samples from group C presented neutralizing activity with median NT_{50} titers of 1:32.0 (T0, range 1:2.8 - 1:81.7), 1:4.0 and 1:3.6 (T1 and T2, range 1:2.3 - 1:6.3). NT_{50} titers were confirmed by positivity in the ELISA assay.
Discussion ad Conclusions: Persistency of anti-SARS-CoV-2 activity was observed up to twenty days after delivery in groups A and B, probably due to the presence of specific neutralizing antibodies in the milk. Interestingly, 63.6% of milk samples collected from group C showed neutralizing activity against the virus and a borderline positivity in the ELISA test, suggesting the possible presence of aspecific antiviral activity. Our study confirms that HBM from infected mothers favors specific immunity in breastfed infants and HBM from non-infected mothers might contain aspecific antiviral compounds, serving as initial protection against SARS-CoV-2 infection.

140 - Expression of Human Endogenous Retroviruses (HERVs) in clinical specimens of colon cancer patients

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Expression of Human Endogenous Retroviruses (HERVs) in clinical specimens of colon cancer patients

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Introduction: Human endogenous retroviruses (HERV) are relicts of exogenous retroviral infections, constituting 8% of the human genome. The genomic structure of HERVs is composed of four main genes: group-specific antigen (*gag*), protease (*pro*), polymerase (*pol*) and envelope (*env*). Alteration of HERVs expression has been related to several cancers, but researches regarding HERVs gene expression in colon cancer are currently sporadic. Very few reports investigated the presence of HERVs transcripts in plasmatic extracellular vesicles (EVs).
Materials and Methods: Fifty-seven Italian patients with advanced-stage colon cancer were enrolled. The expression of HERV-H, -K, -P *env* gene, and HERV-K *pol* gene was analysed in the tumor tissues and negative surgical margins and, when possible, in the peripheral blood mononuclear cells (PBMCs). HERVs presence was also evaluated in the plasmatic EVs, collected from 20 patients. Associations among clinical characteristics, such as tumor location, vascular invasion, perineural invasion, and HERVs gene expression levels were analysed.
Results: HERV-H, -K, -P *env*, and HERV-K *pol* expression levels in the tumor tissues, negative surgical margins and PBMCs were similar. HERVs gene were expressed, at low levels, in the plasmatic EVs of 10% (-H *env*), 40% (-K *env*), 15% (-K *pol*), and 25% (-P *env*) tested patients. Considering the tumor tissue samples, significant associations were found between HERV-P *env* expression, and vascular/perineural invasion ($p<0.05$).
Discussion and Conclusions: In our cohort of advanced-stage colon cancer patients, no reactivation of HERVs expression was observed, confirming that HERVs gene overexpression may be associated only to the early phase of cancer pathogenesis, as suggested by previous studies.
As known, cancer-secreted microvesicles influence the tumor microenvironment and support cancer growth and metastasis: HERVs sequences present in the circulating plasma EVs might be transferred from one cell to another, favouring cellular transforming mechanisms. Investigations regarding the use of HERVs expression as markers of prognosis and as targets of adoptive immunotherapy are warranted.

141 - Study of the effects of new small molecules as antiviral agents against SARS-CoV-2

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Study of the effects of new small molecules as antiviral agents against SARS-CoV-2

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Introduction

This work has been carried out under Tuscavir, an interdisciplinary consortium aimed at performing research and providing qualified services for the development of novel broad spectrum antiviral therapies. Tuscavir is founded by Tuscany Region and includes the University of Siena (UNISI), Azienda Ospedaliera Universitaria Senese (AOUP), Azienda Ospedaliera Universitaria Pisana (AOUP), and the University of Florence (UNIFI) as team members. As part of the project, UNISI has developed different antiviral compounds capable of inhibit SARS-CoV-2 infection *in vitro*. These compounds named TUS (1-12) are small molecules that act against different targets of the viral mechanism of replication. Some of these TUS (1, 2, 3, 4, 5, 6, 7) are entry inhibitors, and others (TUS 11, 12, 13, 14) are Kinase inhibitors. Here we investigate the antiviral activity of single or combined compound against SARS-CoV-2

Materials and Methods

Measurement of the cytotoxicity of the molecules was performed by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], a cell-based assay used for screening a library of compounds to determine if the investigational molecules have effects on cell proliferation or show direct cytotoxic effects, eventually leading cell to death. For every compound the IC 50 was calculated. The antiviral activity against SARS-CoV-2 variants of selected molecules was evaluated by limiting dilution assay. The viral titer was calculated by applying the Reed and Muench formula. In this case, the ability of the molecules to inhibit the entry and /or replication of viral particle was revealed by measuring the cytopathic effect (CPE) on the cellular platform. Moreover, difference in the viral genome production were determined by Real time PCR measurements. Vero E6 cells were adopted for both antiviral activity and cytotoxicity assays.

Discussion and Conclusions

The molecules investigated are generally well-tolerated by Vero E6 cells up to a concentration of 50 µM. This concentration was the maximum used to assess antiviral activity. Inhibition of the levels of infection of SARS-CoV-2 variants on Vero E6 in the presence of drugs was evaluated by using different protocols of administration, according to the supposed mechanism of actions. The reduction of CPE and of the viral genome in the supernatants of infected cells demonstrated the ability of some of the compounds to reduce infection.

155 - SEROPREVALENCE OF TORCH INFECTIONS IN WOMEN OF CHILDBEARING AGE OVER THE 2012-2022 DECADE IN SICILY

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SEROPREVALENCE OF TORCH INFECTIONS IN WOMEN OF CHILDBEARING AGE OVER THE 2012-2022 DECADE IN SICILY

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Introduction

TORCH acronym, classically, includes Toxoplasma gondii (TOX), Other, comprising Treponema pallidum (TP), Rubella Virus (RV), Cytomegalovirus (CMV), and Herpes symplex Virus (HSV). TORCH agents are recognized as major pathogens during pregnancy, being important cause of congenital diseases. The high rate of asymptomatic TORCH infections and the absence of effective vaccines, except for anti-RV live attenuate vaccine, kept high the possibility of pathogens vertical transmission to the fetus during pregnancy. Serological screening represents a key instrument to provide information about immunological status of women of reproductive age in order to identify women susceptible to primary infection and contribute to prevention and treatment of congenital infections. The aim of this study was to retrospectively evaluate the serological status (IgG and/or IgM) of childbearing age women attending to AOUP "P. Giaccone" of Palermo, Sicily over almost 10 years to estimate the adherence to TORCH serological screening program and to value the prevalence of women at risk of primary infections.

Materials and Methods

A total of 2359 sera were collected from women of childbearing age (16-46 years) who were referred to the Microbiology Unit of the AOUP "P. Giaccone" University Hospital of Palermo, Sicily, from 10 November 2012 to 1 April 2022, accessing through the ambulatory care services (National Health Care Surveys) or the Gynecology ward prescriptions.

Results

2359 sera were collected from childbearing age women, whose age ranged from 16 to 46 years, with a mean age of 29 ± 6. Most of the samples had been screened for Abs against a single TORCH pathogen, but 20.9% had been concurrently tested for two pathogens (TOX and CMV), while 14% were screened for three agents (TOX, CMV and RV). Therefore 61.2% had been tested for TOX, 58.6% for CMV, 46.9% for TP, 45.1% for RV and 5% for HSV. The overall seroprevalence was 90.5% for HSV (of which 9.5% for HSV2), 81.2% for RV, 72.1% for CMV, 20.9% for TOX and 4.8% for TP. No noteworthy differences were found in seroprevalence among different age groups analyzed (16–25, 26–35, and 36–46 years) and over the study period. Almost 50% of the women were not aware of their global TORCH serological status and that is still high the proportion of women susceptible to primary infection, in our geographic area.

Discussion and Conclusions

The availability of serological data, collected over almost 10 years, allowed to evaluate the adherence to TORCH serological screening in our geographic area and to define the number of women susceptible to primary infection. The results of this study highlight a limited access to complete TORCH screening as almost 50% of the women of childbearing age while almost all subjects investigated their serological status for one or more of the main TORCH agents, underlining that women in Palermo may not be consistently advised to seek serological testing before and during their pregnancies. The results of this retrospective study clearly confirm the importance of seroepidemiological surveillance study to plan the most effective prevention strategies to reduce the risk of congenital infections.

162 - SARS-CoV-2 OMICRON recombinant epidemiology in Northwest of Italy.

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SARS-CoV-2 OMICRON recombinant epidemiology in Northwest of Italy.

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Introduction: As has happened in the past, RNA viruses can recombine. Recombination can be difficult to detect and requires whole-genome sequencing. The study aimed to investigate the molecular epidemiology of SARS-COV-2 strains in the Lombardy Region, to monitor more aggressive strains. The first recombinant SARS-COV-2 Omicron strains were discovered in January 2022 and the spread is constantly monitored globally. Most Omicron recombinants identified to date have BA.1 as acceptor and the breakpoint within ORF1ab and hence preserve Spike protein from BA.2 (e.g., XE, XG, XH, XJ, XK, XM, XN, XP, XQ, and XR): this is not surprising since BA.2 currently outcompetes BA.1. XP is the lone exception, having BA.1.1 (the BA.1 sublineage with R346K mutation) as an acceptor (including Spike) and BA.2 as a donor.

Materials and Methods: Several nasopharyngeal swabs samples tested positive for SARS-CoV-2 presence, and received in the Microbiology Laboratory of ASST Sette Laghi in the period between April to June 2022 were screened for viral typing. The presence of SARS-COV-2 RNA was determined with the SARS-COV-2 AMP Kit Alinity (Abbott) test and typing was achieved by NGS sequencing (Illumina). According to the current regulations, typing was carried out on samples of a) patients hospitalized and immunocompromised; b) travelers from geographical areas at risk; c) subjects positive to the virus after vaccination; d) contacts of subjects positive to known variants; e) subjects from epidemic outbreaks.

Results: A total of 1000 samples were examined for the presence of SARS-CoV-2 RNA, and 177 (0,2%) were subjected to molecular typing analysis. After PANGOLIN analysis, the recombinant results obtained with sequencing showed the following prevalence rates: 6 (3,4%) were recombinant strains in particular 2 XN (1 BA.2 and 1 B.1.1.529), 1 XQ (BA.2), 1 XT (B.1.1.529), 1 XF and 1 XP. The other most detected lineages were OMICRON 21L, 21K, 21L, 21M, 22A,22B, and 20A.

Discussion and Conclusions: Constant monitoring of the distribution and prevalence of SARS CoV-2 variants is of fundamental utility to trace the presence of mutations potentially involved in the pathogenesis and immune escape of the virus. Currently, recombinant strains of SARS-COV-2 are monitored to understand how they behave over time and continued surveillance for SARS-CoV-2 recombinants is needed.

175 - The role of human DDX3 helicase in HSV-2 infection.

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The role of human DDX3 helicase in HSV-2 infection.

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Introduction: The human protein DDX3 is a DEAD box ATP-dependent RNA helicase that regulates transcription, mRNA maturation and translation. DDX3 is also involved in replication of several RNA viruses. Recently, we demonstrated that some DDX3 inhibitors are effective against several viruses with positive sense single-stranded RNA (ssRNA+) genome such as Coxsackie B (CV-B), while they are not effective against viruses with negative sense single-stranded RNA (ssRNA-) genome such as measles virus (MeV) and vesicular stomatitis virus (VSV). In addition, it seems that DDX3 inhibition also impacts on DNA virus replication. To further clarify the role of DDX3 protein in viral infection, we investigate the effect of DDX3 inhibitors during Herpes Simplex virus 2 (HSV-2) infection. HSV-2 is a double strand DNA (dsDNA) virus belonging to Herpesviridae family. HSV-2 is a human pathogen and it is primarily associated with genital lesions but is also responsible for severe infections in both immunocompetent and immunodeficient individuals. Replication of HSV-2 occurs in the cell nucleus, where the virus takes over the host gene expression machinery during active infection. The compounds we tested target specifically DDX3 RNA binding domain leaving the ATP-binding domain unchanged.

Materials and Methods: A549 cells were infected with 0.004 MOI of HSV-2 in the presence of DDX3 inhibitors and monitored up to 2 days post infection (DPI). Antiviral activities of these compounds were determined *in vitro* by plaque assay and the half maximal inhibitory concentration (IC_{50}) and Selectivity Index (SI) were calculated. To evaluate the production of DDX3 and viral proteins, western blotting was performed by infecting A549 cells with 0.1 MOI of HSV-2 and doing protein extraction at different hour PI. Furthermore, A549 cells were infected with 0.1 MOI of HSV-2 in presence of DDX3 inhibitors and another western blotting was carried out 2DPI. Moreover, molecular analyses were performed to evaluate DDX3 and viral gene expression. Lastly, distribution and localization of DDX3 during HSV-2 infection were visualized by immunofluorescence.

Results: During the infection of cells with HSV-2, the level of DDX3 protein is significantly reduced, its cytoplasmatic distribution is altered compared to uninfected cells and DDX3 protein may colocalize with gD 1/2 HSV-2 protein. As DDX3 inhibitors exert antiviral activities against HSV-2, they are able to restore DDX3 protein level and cellular localization.

Discussion and Conclusions: DDX3 seems to be involved in HSV-2 infection; for this reason, it may be an excellent target to develop antiviral agents clinically relevant against HSV-2 infection.

184 - Antiviral activity of natural compounds and composite polymeric matrix against SARS-CoV-2.

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Title: Antiviral activity of natural compounds and composite polymeric matrix against SARS-CoV-2.

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Introduction: The ongoing SARS-CoV-2 pandemic is pushing for the development of better and more protective personal protective equipment (PPE) to ensure greater safety in use. Metallic nanoparticles (NP), particularly copper or silver, have shown antibacterial and antiviral potential but also noticeable cell toxicity. Combining metal NP with a composite polymeric matrix could maintain the antiviral capacity and reduce toxicity due to NP dispersion. A further increase in virucidal capacity could be obtained by combining NP and essential oils such as the flavonoids that are known for their antiviral properties. Here, specific composite matrices, some of which have already been tested against influenza virus, hepatitis virus and immunodeficiency virus, were probed against SARS-CoV-2.

Materials and Methods: The nanomaterial composites in study were produced by IFC-CNR and IBF-CNR. Antiviral activity was tested in Vero-E6 cells using a clinical strain of SARS-CoV-2. Various concentrations of different flavonoids were tested to assess cytotoxicity. The compounds were incubated at different concentrations and different times with a titrated amount of SARS-CoV-2 and then seeded on Vero-E6 cells. Similarly, nanomaterials layered on discs of a polyurethane matrix were left in contact with the virus at different time points and the viral suspension was then added to Vero-E6 cells. In both experimental settings, cytopathic effect and viral release in supernatant were measured after three days of incubation.

Results: Both Quercetin and Myricetin did not show a cytotoxic effect up to 100 microM. Both compounds exhibited a dose-dependent virucidal effect that reached 90% and 69% reduction with 50 microM of Quercetin and Myricetin respectively. Ag-NP composite nanomaterial exerted an antiviral effect of nearly 60% after one minute of incubation and up to 90% after 10 minutes. Cu-NP composite nanomaterial showed an even higher effect that reduced the viral titer by 80% and 99% after 1 and 10 minutes, respectively.

Discussion and Conclusions: Polyurethane composite nanomaterials have shown good virucidal properties, while flavonoids appear good candidates to develop natural antivirals. Developing PPE coatings composed of NP embedded in a polymeric matrix could improve their efficacy. These new materials could coat many different surfaces and be active against multiple microorganisms.

185 - Palmitoylethanolamide (PEA) inhibits SARS-CoV-2 entry by interacting with S protein and ACE-2 receptor.

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Title: Palmitoylethanolamide (PEA) inhibits SARS-CoV-2 entry by interacting with S protein and ACE-2 receptor.

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Introduction: The rearrangement of lipid membranes is a crucial step in the replication of several positive-strand RNA viruses such Coronaviridae. Due to the close connection between lipids, inflammation and viral infection, lipid metabolism has become an area of intense research.
Our work investigates the antiviral potency of PEA, an endogenous lipid mediator with analgesic and anti-inflammatory activity mediated by the activation of peroxisome proliferator receptor alpha (PPAR-α). This lipid was selected among hundreds of compounds for its in-silico predicted ability to bind SARS-CoV-2 S protein. This work aims to validate the in-silico prediction and assess how PEA might act as antiviral and antinflammatory compound during COVID19 pathogenicity.
Materials and Methods: Infection of Huh-7 and Calu-3 cells was performed with 0.1 MOI of SARS-CoV-2 VR PV10734, B.1.617.2, and B1.1.529. Micronized PEA was used at various concentrations to treat cells prior to the infection or in a solution with the viral strains. Neutralization assays were used to validate the entry inhibition, while qRT-PCR and immunocytochemistry were used to assess infection rates.
Results: Preliminary *in silico* analyses predicted the interaction of PEA with SARS-CoV-2 S protein. Our results confirmed this prediction, while PEA causes a reduction of viral infection by nearly 70% when administered to cells or incubated with SARS-CoV-2 virions prior to infection. Interestingly, PEA antiviral activity was maintained among all SARS-CoV-2 variants. Then, we demonstrated by Cell-based ELISA assay the interaction between PEA and the RBD domain of Spike protein.
Finally, we discovered that PEA administration drops Lipid Droplets content in treated cells by 40%. These vesicles are the very same used by SARS-CoV-2 to boost its replication and enhance inflammation.
Discussion and Conclusions: Two different mechanisms could explain the activity of PEA: the inhibition of viral entry caused by PEA binding on SARS-CoV-2 S protein and the activation of lipolysis that dismantles lipid droplets, a fundamental resource of energy and protection for the virus.
The present work demonstrates a novel mechanism of action for PEA as a direct and indirect antiviral agent against SARS-CoV-2.
Further preclinical and clinical tests will be needed to fully consider this lipid as a promising adjuvant therapy in the current COVID-19 pandemic or against emerging RNA viruses that share the same route of replication as Coronaviruses.

193 - First identification of SARS-CoV-2 Omicron BA.4 sub-variant in Italy: genomic comparison with omicron sub-variants

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First identification of SARS-CoV-2 Omicron BA.4 sub-variant in Italy: genomic comparison with omicron sub-variants

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Introduction

We reported the first case of SARS-CoV-2 omicron variant in our region (Calabria, Italy) on December 5, 2021. The Omicron variant of Concern (VOC), has rapidly replaced the delta variant as a dominating SARS-COV-2 variant because of natural selection, favouring the variant with higher infectivity and more strong vaccine breakthrough ability. BA.5 and BA.4 lineages are the currently prevailing sub-variant, herein described and first detected in Italy, on April, 25 by depositing it in ICOGEN Platform by Istituto Superiore di Sanità (ISS). The omicron lineage BA.4 is here studied in comparison with the other three lineages, together with the capability of five different type of Rapid Antigenic Tests (RATs) to recognize it.

Materials and methods

A positive nasopharyngeal and oropharyngeal swab was collected in UTM™ and extracted for viral nucleic acids purification. The Real Time (RT) PCR test was carried out with the TaqPath COVID-19 CE-IVD RT-PCR kit, which targets the following genes of SARS-CoV-2: i) open reading frame (ORF)1ab; ii) nucleocapsid (N) and iii) spike (S). RNA extracted by the specimen, underwent genomic characterization following two methodologies: the Sanger-based sequencing and whole-genome based on next-generation sequencing (NGS) by MiSeq System. CleanPlex SARS-CoV-2 FLEX Paragon Genomics Panel performed a reverse transcription of the whole-genome and library preparation. The SOPHIA Platform analyzed FASTQ reads. Clade analyses were obtained by ICOGEN Platform (ISS) and the GISAID database.
Alignment of FASTQ obtained by NGS data were performed SnapGene® software. SNAP gene finder has been developed so far to be easily adaptable to a variety of genomes.

Results
The NGS approach and subsequent analysis of FASTQ reads by SOPHIA DDM Paltform, revealed BA.4 omicron variant. The sequences were deposited in the ICOGEN Platform on the April, 25 2022 and GISAID on the April, 26 2022.The bioinformatics comparison analysis with the other three sub-variants, pointed out a new mutation specifically a deletion in ORF 1 ab gene which correspond to KSF141_del in non-structural protein 1 (nsp1) a critical virulence

factor able to eskape immune-response. This deletion was not present previously and therefore was never reported till now. Concerning the Rapid Antigenic Tests (RATs) assayed, we obtained a positive result for all five antigenic tests evaluated.

Discussion and Conclusions

Our data showed a new deletion in the ORF1 ab gene linked to nsp1 protein that was indicating as an interesting target for the development of live attenuated vaccines with deletions in key regions of nsp1. For this reason, is necessary to study the viral strain by NGS approach in order to discover novel mutations and develop more effective vaccines.

195 - In vitro virucidal activity of active food packaging based on monoglycerides against a Human Coronavirus and other viruses

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In vitro virucidal activity of active food packaging based on monoglycerides against a Human Coronavirus and other viruses

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INTRODUCTION

The ongoing pandemic by SARS-CoV-2 has generated an increased demand for materials with virucidal properties, also in the field of food packaging. A solid body of evidence documents the microbicidal activity of lipids, such as fatty acids and monoglycerides, on a large spectrum of microorganisms. In this work, virucidal activity of four different formulations based on monolaurin and monocaprine, were tested on betacoronavirus HCoV OC43, Herpesvirus HSV-1 and the Adenovirus AdV-5. The first aim was to assess the most active formulation against these viruses. In a next step, the best performing formulation was used as antimicrobial agent to develop a film as active food packaging.

MATERIALS AND METHODS

Each of the 4 lipid formulations was tested at different concentrations and times of contact by incubating the virus suspensions with each formulation for 10 and 20 minutes followed by titration with end point dilution. In a second phase the most active formulation was used to prepare a packaging film which was tested for its ability in inactivating HCoV OC43 and HSV-1 but not AdV which resulted not invactivated by any of the 4 formulations. The virucidal activity of this active packaging was tested at different concentrations and time of contact, up to 24 hour. Briefly, the films were contaminated with the viruses under study and at different time points the film samples were soaked in 1ml of cell growth medium and vortexed for 1' to eluate the survived virus. Viral loads of these suspensions were titrated by end point dilution on cell cultures.

RESULTS

All the four formulations tested were found active on HCoV OC43 and HSV-1 (in some cases with a reduction >4 Log) but not on AdV-5. The packaging film showed a remarkable reduction of the viral load of both virus (>2,5 Log).

DISCUSSION

These results confirm that natural compounds with antiviral properties for active food packaging may represent a new and promising strategy for virus transmission.

204 - Gold-Nanoparticle delivery of CRISPR/Cas13 as antiviral strategy against emerging RNA viruses

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Gold-Nanoparticle delivery of CRISPR/Cas13 as antiviral strategy against emerging RNA viruses

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Introduction: The pandemic that started in 2019 due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) highlighted the urgency of novel therapeutic approaches that can easily be used against emerging viruses whereas no vaccines or therapies are available. Recent evidence show that the enzyme CRISPR Associated Protein (Cas) 13 is capable to destroy SARS-CoV-2 genomes in infected cells. Cas13 localizes in the very same organelles in which both SARS-CoV-2 and ZIKV replicate and is capable to abolish infection in transfected cells. Unfortunately, no effective delivery of this protein is available at the moment. The aim of the present work is to use nanoformulated nanoparticles (GNPs) to deliver Cas13 into infected cells without any kind of transfection protocol and evaluate its effectiveness of it as a new efficient antiviral approach.
Materials and methods: *Selection of gRNAs*: We obtained HuH-7 lines stably expressing Cas13 and one gRNA previously selected, targeting N/RdRP and NSP3/C genes in SARS-CoV-2 or ZIKV genomes, respectively. These cells were then infected. Then, intracellular and extracellular viral load was detected by q-RT-PCR. The antiviral potency of GNP-Cas13 was then validated by Western Blot and Immunocytochemistry assays.
Evaluation of localization and efficacy of nanoformulation: we functionalized GNPs with Cas13 enzyme. Then, GNP-Cas13 were used to evaluate the cellular localization by confocal live imaging assays. Finally, GNP-Cas13 directed against SARS-CoV-2 N and ZIKV C proteins were administered to Huh-7 and Calu-3 cells before or after viral infection.
Results: *Selection of gRNAs:* We observed a reduction of SARS-CoV-2 infection when targeting RNA-dependent RNA polymerase (RdRP) and Nucleocapsid (N) protein resulting as if they had not been infected. In agreement, we observed the same reduction when ZIKV NSP3/C protein was targeted.
Evaluation of localization and efficacy of nanoformulation: our nanoformulation spontaneously enter in cells and localize into cytoplasmatic vesicles (autophagosomes, endosomes). In agreement, we observed a 3-logarithm decreased number of infected cells when GNP-Cas13 are administered before infection.
Discussion and conclusions: The results confirm that the CRISPR/Cas13 system effectively degrades the genomes of ZIKV and SARS-CoV-2 and blocks the infection when proper genomic sites are targeted. At the same time, GNPs are an excellent delivery system, as they allow the Cas13 to enter cells spontaneously and localize in the very same organelles in which these viruses replicate.

207 - Humoral and cell-mediated immune response in COVID-19 vaccinees according to history of prior SARS-CoV-2 infection.

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Humoral and cell-mediated immune response in COVID-19 vaccinees according to history of prior SARS-CoV-2 infection.

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Introduction: The present study aimed to monitor humoral and cell-mediated immune response to COVID-19 vaccines according to history of prior SARS-CoV-2 infection. To this purpose healthy adults and frail nursing home (NH) residents were enrolled at time of their first vaccination.
Material and Methods: Anti-trimeric Spike (Anti-S) IgG antibody levels were assessed at study enrolment (prior to the first dose of vaccine, T0) and then after 2 (T1), 6 months (T2) and 12 months (T3). Between T2 and T3 subjects received a third booster vaccine dose.
T-cell responses were measured at T2 and T3 in NH residents, as the frequency of Spike-specific $CD4^+$ and $CD8^+$ T cells producing IFN-gamma, TNF-alfa and IL-2 assessed by flow cytometry.
Results: Anti-trimeric Spike IgG were induced by the vaccination and their levels decreased in the interval between T1 and T2. Superior humoral immunity was induced in healthy adults and NH residents with a previous SARS-CoV-2 infection. Twelve months after the first vaccination (T3) the levels of Anti-S IgG were substantially increased both in healthy adults and in NH residents, and the effect of previous SARS-CoV-2 infection was no longer apparent.
Superior cell-mediated immunity was evident at T2 in NH residents with a previous SARS-CoV-2 infection. Similar levels of T-cell responses were observed at T3 in residents with previous SARS-CoV2 infection, while an increase in Spike-specific T cells was observed between T2 and T3 in COVID-19 naïve subjects, especially increasing the $CD8^+$ response.

Discussion and Conclusions: These preliminary results show that a previous SARS-CoV-2 infection significantly potentiates the immune response to vaccination up to six months after the infection. Overall, these findings might provide important information for future prevention strategies.

226 - Antiviral activity of fungal secondary metabolites against Canine coronavirus infection

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Antiviral activity of fungal secondary metabolites against Canine coronavirus infection

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Introduction - Canine coronavirus (CCoV-II), an alphacoronavirus, may cause self-limiting enteric disease in dogs, especially in puppies. The noteworthy plasticity of CoVs occurs through mutation and recombination processes, which occasionally generate new dangerous variants. The current SARS-CoV-2 pandemic as well as the recent detection of a novel canine-feline recombinant alphacoronavirus isolated from a human patient emphasizes the cross-species transmission ability of CoVs. In this contest, studying antiviral compounds is considered essential to fight CoVs infections.

Fungi produce secondary metabolites (SMs), often developed as antibiotics, fungicides, hormones, and plant growth regulators. Screening performed on benzo-γ-pyrone 3-*O*-methylfunicone, a SM produced by *Talaromyces pinophilus*, showed that it reduces infectivity of hepatitis C virus and bovine herpesvirus 1. Based on this evidence, herein antiviral ability of OMF was evaluated against CCoV-II infection.

Materials and methods - OMF was obtained by extraction and chromatographic purification of culture filtrate of *T. piniphilus* (strain LT6). Following CCoV (378/strain) infection in canine fibrosarcoma (A72) cell line, bioscreen, immunofluorescence staining, cytomorphological and virus yield analyses were performed.

Results - During CCoV infection, the non-toxic concentration of 0.5 µM OMF markedly increased signs of cell viability. Moreover, OMF induced a significant reduction in virus yield. These findings occurred in the presence of an intense downregulation in the expression of the viral nucleocapsid protein.

Discussion and Conclusions - Taken together, our results suggest that OMF shows a potential activity against CCoV infection. To date, very few antiviral compounds to fight CCoV infection have been described. The screening of hypothetical antivirals in *in vitro* animal model of CoVs avoids the manipulation of extremely dangerous human CoVs (SARS-CoVs, MERS-CoV).

227 - Canine coronavirus activates the aryl hydrocarbon receptor during infection

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Canine coronavirus activates the aryl hydrocarbon receptor during infection

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Introduction - The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that interacts with various endogenous and exogenous substrates such as bilirubin, biliverdin, tryptophan metabolites, environmental pollutants, as well as microbial metabolites. After the activation, AhR translocates into the nucleus where it controls the expression of target genes, including AhR repressor, detoxifying monooxygenases, and cytokines. Current findings establish that AhR signaling can influence intrinsic, innate and adaptive immune response to different microorganisms. Recently, it has been demonstrated a role for AhR as a controller of the host response to Coronaviruses (CoVs) (MCoV, SARS-CoV-2, HCoV 229E) infection. Genotype II of canine coronavirus (CCoV-II), an alphacoronavirus, can provoke moderate to severe enteric disease in dogs. Herein, we tested the involvment of AhR in CCoV infection.

Materials and methods - Infection of CCoV (378/strain) in canine fibrosarcoma (A72) cell line was carried out in the presence of CH22319149, an AhR antagonist. Bioscreen, immunofluorescence, and virus yield analyses were performed.

Results - During CCoV infection an upregulation of AhR was detected. The non-toxic concentration of 2 □M CH22319149 markedly decreased cell death signs and increased cell viability. Furthermore, the AhR antagonist provoked a considerable decrease in virus yield. These results were accompanied by the inhibition of the expression of viral nuclear protein.

Discussion and Conclusions - Overall, our findings demonstrate that infection with CCoV activates AhR. In addition, pharmacologic AhR inhibition provokes a reduction in CoVs replication *in vitro*, identifying AhR as a conceivable candidate target for antiviral therapy.

236 - In vitro study of the interaction of enoxaparin sodium or heparan sulfate with not SARS-CoV strains

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In vitro study of the interaction of enoxaparin sodium or heparan sulfate with not SARS-CoV strains

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Introduction: As is now well known, the entry into human cells of the SARS-CoV and SARS-CoV-2 viruses is initiated by binding to angiotensin converting enzyme 2 (ACE2). In fact, the attachment of the virus to the host cell is initiated by the interactions between the protein S and its receptor. Anyhow, the site of receptor binding domains within the S1 region of a coronavirus protein S varies for each coronavirus. Additionally, the spike protein of other human coronaviruses can bind to a secondary receptor, or co-receptor, to facilitate such entry. (MERS)-CoV uses sialic acid as a co-receptor together with its main receptor dipeptidyl peptidase 4. Human CoV-NL63, which also uses ACE2 as a receptor, uses heparan sulfate (HS) as a coreceptor. The SARS-CoV pseudovirus also uses HS as a co-receptor for infectivity. Differently, HCoV-229E uses human aminopeptidase N while HCoV-OC43 uses 9-O-acetyl-sialic acid. The aim of this work was to investigate the possible interactions between enoxaparin sodium salts (EX) and heparan sulfate (HS) on Coronavirus strains that do not have ACE2 as the main interaction receptor (HCoV-229E and HCoV-OC43) in order to be able to highlight these molecules as inhibitors of viral adsorption. **Materials and Methods**: The cytotoxicity of these molecules on MRC5 or HCT-8 cells was evaluated at different times by MTT. Moreover, an air-liquid interface (ALI) exposure using a Borgwaldt LM4E vaping machine with an aerosol nebulizer attached for the vaporization of the solutions object of our study directly on the cellular monolayer, was also investigated. Finally, for the evaluation of the antiviral activity, experimental condition was performed by co-exposure of HCoV-229E and HCoV-OC43 with HS and EX. **Results:** Both EX and HS showed cytotoxicity in a dose depending manner both in normal treatment or ALI exposure. Both EX and HS showed a slight viral inhibition. In fact, a reduction in virus CPE of about 30% was observed with the tested dose equal to 2.5 mg/mL against 229e. All other doses tested showed no inhibition, as well as, no inhibition was observed for HCoV-OC43 strain at any of the doses tested. **Discussion:** It is known that, SARS-CoV-2 spike protein interacts with glycosaminoglycans such HS to enhance the attachment of viral particles to the cell surface by promoting their entry, conversely EX has been reported to negatively interact with virus adsorption (SARS-CoV-2). Our results demonstrated that they could also interact with other cellular receptors for coronavirus for this reason other *in vitro* experiment and molecular docking modeling are on-going.

238 - Identification of interactions between human nuclear proteins and HPV16 genome

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Identification of interactions between human nuclear proteins and HPV16 genome

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Introduction: Human Papillomavirus (HPV) is a member of the *Papillomaviridae* family, characterized by infecting cells in active proliferation such as epithelial cells, and its replicative cycle is associated with the differentiation of the host cell. HPV infections are one of the most common causes of sexually transmitted diseases spread around the world; it is estimated that about 75% of people contract the virus at least once in their lives: the infection affects both women and men, especially in the juvenile age, which coincides with the beginning of sexual activity. HPV is present in 99.7% of cervical cancers and in a high percentage in anal carcinomas, but vaginal, vulva and oral carcinomas are not excluded. To date, over 200 different genotypes are known, each of which shows a narrow tissue specificity for infection. Globally, almost 70% of cervical carcinoma cases are caused by HPV 16 and 18, while the remaining 30% is caused by HPV 31, 33 and other genotypes.

To counter this viral spread, it is advisable to increase prevention through vaccination, in fact, to date there are 3 vaccines approved by the Food and Drug Administration (FDA) as there is no effective treatment for cancer caused by HPV**.** The aim of this project is to identify all human proteins that interact with the HPV 16 genome in order to find new therapeutic drugs, through the use of an innovative approach. To date, several techniques have been known to detect such interactions as the chromatin immunoprecipitation test (ChIP) and the DNA pull-down test, both presenting limitations. Then an evolution of DNA pull-down was carried out for long regions of DNA modified with biotinylated oligo, thus identifying a new method: Long Regions of DNA Pull-down (LDP). **Materials and Methods:** First, hybrid oligos were designed using Primer 3 software for the entire HPV 16 sequence genome. Plasmid phpv-16 in the bacterium E.coli (ATCC" 45113~) was amplified, extracted and used as a template for the first stage amplification by

PCR pull-down. The first PCR amplifies the region of interest with the use of hybrid primers that carry a specific region and a second unique region not present in the genome. The 500 bp amplified segments, thus obtained, were used as templates in the second phase of PCR, to obtain fragments with biotinylated ends in 5'. In the meantime, the nuclear extraction of SiHa cells (Elabscience EP-CL-0210), containing about 3 integrated copies of HPV 16 per cell, has been conducted. Subsequently, the DNA pull-down was carried out: the biotinylated dsDNA fragment interacted with the streptavidin sepharose beads and the nuclear cell extract was added to this complex, and, finally, the final product was analyzed using Liquid Chromatography Mass Spectrometry-Mass Spectrometry (LC/MS-MS). **Results:** About 4350 proteins were identified by mass spectrometry analysis and 310 of these were unique. Gene ontology (GO) was carried out by analyzing the biological processes, molecular functions and cellular components in which the identified interactors participate. An important part of them is involved in DNA replication, transcription and translation. **Discussion and Conclusions:** This technique could offer new informations about HPV 16-host cell interaction, for example new interactions involved in the mechanism of integration of the virus in the host cell which are still unknown. Further studies need to be conducted to investigate new therapies to combat the infection, progression and pathogenicity of HPV 16.

240 - Antiviral Activity of the Rhamnolipids from the Antarctic Bacterium Pseudomonas gessardii against Herpesviridae and Coronaviridae family

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Antiviral Activity of the Rhamnolipids from the Antarctic Bacterium Pseudomonas gessardii against Herpesviridae and Coronaviridae family

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Introduction: Emerging and re-emerging viruses pose a serious threat to human health, consequently, new strategies are required to counteract their development. In this context, rhamnolipids (RLs) are good candidates. RLs are a class of glycolipids produced by the genus *Pseudomonas*, consisting of one or two rhamnose linked to one of two fatty acid chains. RLs show a several advantages compared to synthetic surfactants; they are ecological, non-toxic and biodegradable. The RLs studied in this work were produced by an Antarctic *Pseudomonas gessardi* bacterium and evaluated for their antiviral action against the *Coronaviridae* and *Herpesviridae* family.

Material and method: To evaluate the antiviral activity of RLs, plaque reduction assay was performed. RLs activity was assessed against the enveloped viruses Herpes simplex virus type 1 (HSV-1) and 2 (HSV-2), Human coronavirus strain 229E (HCoV-229E), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and against the naked Poliovirus Type 1 (PV-1). The action of the RLs on the viral envelope was observed with transmission electron microscope (TEM). Moreover, the ability of RLs to inactivate viruses from surfaces was demonstrated by preparing 12-well plates coated with RLs. Finally, the cytotoxicity was evaluated by methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay on Vero and HaCaT cell lines.

Results: Our results show complete inactivation of HSV-1 and HSV-2 by RLs at 6 µg/mL, and of HCoV-229E and SARS-CoV-2 at 25 and 50 µg/mL, respectively. No activity against PV-1 was detected, suggesting that the antiviral action is mainly directed towards the envelope. TEM images validated this hypothesis, showing HSV-1 particles lacking both tegument and envelope when treated with RLs. On the other side, TEM images showed that the main results of RLs action on SARS-CoV-2 is the detachment of S proteins from the envelope. Finally, the MTT assay at 100 µg/mL, Vero and HaCaT cells showed 80% and 45% of viability, respectively. The RLs showed no toxicity for both cell lines used at the other tested concentrations.

Discussion and Conclusions: RLs represent one of the most promising compounds in the pharmaceutical industry because of their structural versatility, stability and low toxicity that are useful in the design of therapeutics. The high antiviral activity of RLs encourage the application of these biosurfactants to counteract the widespread of viral

pathogens. The results suggest the opportunity of using RLs as antiviral additives in formulations of hand sanitizers, anti-herpes lipsticks and intimate detergents, surface and household cleaners, and as antimicrobial agents.

241 - Evaluation of HPV genotype-specific viral loads in clinician-collected cervical samples as compared to vaginal self-collected samples in women with cervical dysplasia

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Evaluation of HPV genotype-specific viral loads in clinician-collected cervical samples as compared to vaginal self-collected samples in women with cervical dysplasia

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Introduction

According to European Guidelines many countries, including Italy, are switching to Human Papillomavirus (HPV)-based primary cervical cancer screening. The introduction of HPV test offers the possibility to use self-sampling as a valid well-accepted alternative to improve screening participation. PCR-based HPV tests on self-samples provide similar accuracy as cervical samples in the detection of cervical pre-cancer lesions.

The aim of this study was to assess agreement and compare type-specific high-risk HPV (hrHPV) viral load in paired vaginal-self and clinician-collected cervical samples using a quantitative full-genotyping hrHPV assay.

Materials and Methods

As part of a prospective clinical study conducted according to the European VALHUDES protocol (ClinicalTrials.gov Identifier: NCT04312737), 600 women were enrolled in 4 colposcopy centers. Prior to gynecological examination, women were asked to collect a self-vaginal swab using FLOQSwab® (Copan), while the clinician collected a cervical scraping with Cervex-Brush (Rovers). Cervical samples were immediately suspended 20mL ThinPrep® (Hologic), while vaginal self-collected swabs were transported dry to the laboratory and there suspended in 5ml of ThinPrep® (Hologic) or eNat® (Copan). A totally automated platform (Fluent 480, Tecan) was used for nucleic acid extraction using *Quick* DNA/RNA viral MagBead (Zymo) and Real-Time PCR preparation with HPV OncoPredict QT assay. HPV OncoPredict QT is a quantitative assay that individually detects and quantifies 12 hrHPV and sample cellularity. Viral load was normalized to sample cellularity and expressed as viral copies/cells. Kappa values (k) and Mann-Whitney tests were used to assess test concordance and differences in median viral load between samples.

Results

Eighty-eight percent of cervical and vaginal-self samples had a concordant result in hrHPV detection (k=0.75, 95%CI: 0.69-0.81). Individual HPV genotype concordance was moderate and good (k ranging from 0.64 to 0.93).

Type-specific viral loads were significantly higher for all individual genotypes in cervical compared to vaginal samples (Table 1). Interestingly, HPV51 and HPV58 median number of viral copies/cells were higher compared to other genotypes in both sample types.

The median number of cells in vaginal specimens (36,456 [IQR 1,8181-52,359] was significantly higher than in cervical samples (3,410 [IQR 1,180-8,524]).

Discussion and conclusions

The agreement in hrHPV detection between clinician-collected cervical and vaginal-self samples was good. Cellularity was higher but viral loads were substantially lower in vaginal compared to cervical samples.

Table 1: Type-specific median viral load expressed as viral copies/cells [IQR] of 12 hrHPV in cervical and vaginal-self samples.

HPV genotypes	N°	Cervical samples Median viral load [IQR]	Vaginal-self samples Median viral load [IQR]	p-value[a]
HPV16	98	1.47 [0.15-15.39]	0.02 [0.00-0.19]	0.0000
HPV18	19	0.36 [0.02-17.63]	0.01[0.00-0.07]	0.0003
HPV31	67	4.14 [0.07-26.37]	0.05 [0.01-0.57]	0.0000
HPV33	25	2.53 [0.20-37.56]	0.01 [0.00-0.35]	0.0000
HPV35	13	0.42 [0.04-4.75]	0.004 [0.00-0.04]	0.0015
HPV39	31	0.59 [0.02-9.59]	0.02 [0.00-0.35]	0.0001
HPV45	19	0.02 [0.00-1.55]	0.001 [0.00-0.04]	0.0019
HPV51	43	47.87 [1.00-438.50]	1.51 [0.07-17.69]	0.0000
HPV52	44	0.67 [0.03-1.67]	0.02 [0.00-0.21]	0.0000
HPV56	44	1.44 [0.02-17.54]	0.06 [0.00-0.64]	0.0000
HPV58	29	31.64 [5.52-356.08]	0.38 [0.03- 6.45]	0.0000
HPV59	24	0.51 [0.02-13.72]	0.05 [0.00- 3.31]	0.0010

247 - Prion-like alpha-synuclein detection in ante-mortem intestinal biopsies from Parkinson's disease patients by RT-QuIC assay

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Prion-like alpha-synuclein detection in ante-mortem intestinal biopsies from Parkinson's disease patients by RT-QuIC assay

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Introduction: Aggregation of misfolded alpha-synuclein (alpha-Syn) has been described as one of the most promising biomarkers of Parkinson's disease (PD). In recent years, the concept that misfolded alpha-Syn can spread from cell-to-cell likewise to infectious prion agents has increasingly gained agreement in the field. In fact, several reports have established that PD can be experimentally transmitted by a prion-like mechanism in different cellular and animal models. These findings have relevant implications for the applicability of innovative diagnostic approaches based on the prion principle of spreading by seeding to amplify the pre-existing seeds in vitro. Real-time quaking-induced conversion (RT-QuIC) assay is a breakthrough approach based on prion-like seeding activities recently exploited for the detection of seeds of alpha-Syn in different specimens from PD patients. The gastrointestinal (GI) tract has been proposed as a possible site of origin of alpha-Syn aggregation suggesting that the detection of a-Syn in the GI has high potential as in vivo and predictive diagnostic biomarker of PD. Previous studies showed alpha-Syn seeding activity in colon samples from rodents and post mortem biopsies of PD by RT-QuIC assay. In the present study, we evaluated for the first time the suitability of RT-QuIC assay for the ante-mortem detection of alpha-Syn in intestinal mucosa biopsies from patients with a clinical stage of PD.

Methods: A total of 24 participants to this study, 20 PD patients diagnosed according to the UK Brain Bank criteria, and 4 non-neurodegenerative healthy controls subjects were recruited. All patients underwent upper GI endoscopy for placement of an administration jejunal extension tube (PEG-J) for continuous levodopa enteral infusion, contextually. Two GI biopsies were taken for each patient. Based on the rostrocaudal distribution of alpha-Syn in the GI tract, the proximal small intestine (duodenum) was chosen as the most appropriate part for the analysis. A mutant human recombinant of alpha-Syn was used as substrate for the RT-QuIC reactions.

Results: alpha-Syn RT-QuIC analysis showed positive reactions in 17/20 patients with PD and negative reactions in all other non-neurodegenerative healthy subjects. RT-QuIC assay achieved a diagnostic sensitivity of 85% and specificity of 100%.

Discussion and Conclusions: These findings highlight for the first time that RT-QuIC reaction allows a sensitive detection of alpha-Syn seeds in safely accessible ex vivo intestinal biopsies from PD patients. The potential use of ultrasensitive RT-QuIC technique for the detection of very small amounts of intestinal alpha-Syn from live PD patients open a way for improved *intra vitam* diagnosis of clinical and prodromal PD.

252 - Inhibition of viral entry by peptides designed on the glycoproteins Gn and Gc of Schmallenberg virus

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Inhibition of viral entry by peptides designed on the glycoproteins Gn and Gc of Schmallenberg virus

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Introduction. In recent years arthropod-borne viruses are emerging as a serious threat to human and animal health worldwide. Among the Orthobunyaviruses, the Schmallenberg virus (SBV) represents a novel and major member, appeared in central Europe during the summer of 2011 and rapidly spread all over Europe. The virus affects mainly but not exclusively the domestic ruminants causing abortion and malformation in the offspring, as well as febrile episodes, decreased milk production and diarrhea in adults. The viral particle is enclosed in a membrane formed by the glycoproteins N (Gn) and C (Gc) able to mediate the viral entry by means of their fusogenic potential. The fusion peptide is located on Gc, a class II fusion protein, but cell fusion requires the expression also of Gn, a chaperone essential for the correct trafficking of Gc to the Golgi complex before viral budding. Therefore, both Gn and Gc glycoproteins may represent a target for antiviral development. In the present study, we investigated the inhibitory activity mediated by synthetic overlapping peptides designed on the amino acid sequences of the proteins. **Materials and Methods**. We have synthesized peptides by standard 9-fluorenylmethoxycarbonyl polyamine solid-phase synthesis. Peptides cytotoxicity were evaluated on hamster kidney cells (BHK-21) at different concentrations starting by 100 µM via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Then cells were simultaneously treated with each peptide at 100 µM and infected by SBV (gently provided by University Federico II of Naples). To better understand how peptides could interfere with the SBV lifecycle, different time of addition and temperature shift assays were performed at scalar concentrations. Furthermore, the expression levels of the viral M gene were analyzed. Finally, the secondary structure of peptides was determined via circular dichroism (CD). **Results**. We have performed a brute analysis of both glycoproteins in order to explore the inhibitory activity of such peptides against SBV infection. Five out of the Gc peptides at a concentration of 100 µM reached the 50% of inhibition arbitrary cut-off. None of the Gn peptides had a consistent inhibiting effect and no peptide toxicity was observed by the MTT assay at the concentrations used in our experimental conditions. Gc peptides were able to target the viral particles, by preventing the early stages of the infection, namely virus attachment and entry into the host cell. In addition, molecular tests indicated that the two most active peptides (Gc30 and Gc49) were also able to interfere with gene expression. Finally, we analyzed their secondary structure via CD, revealing that Gc30 had a prevalent α-helical structure, differently from Gc49, which adopted a prevalent β-structure. **Discussion and conclusions.** Our data indicate the possible direct involvement of Gc described domains in the process of virus penetration; therefore, these results are of relevance to the potential development of novel therapeutic

compounds to prevent SBV infections and could serve as a model for many human pathogens belonging to the same family.

256 - A Cluster of SARS-CoV-2 omicron variant of concern harbouring a rare mutation in the spike gene

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Title: A Cluster of SARS-CoV-2 omicron variant of concern harbouring a rare mutation in the spike gene

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Introduction: Whole-genome sequencing (WGS) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) helps distinguishing hospital-acquired from community-acquired infection by identifying the presence of clusters with common mutation patterns. In April 2022, 4 patients admitted to the Internal Medicine Unit of the Pontedera Hospital contracted SARS-CoV-2 infection during their hospitalization. Their clinical outcome was characterized by dry cough, hypophonia, and low-grade fever. The nasopharyngeal swabs (NPS), collected during the acute phase of the disease, were sent to the Virology Unit of the Azienda Ospedaliera Universitaria Pisana to search for SARS-CoV-2 genome and, if present, characterize the viral variant.
Materials and methods: The extracted RNA from NPS was processed for WGS by using Illumina COVIDSeq Assay kit and sequenced on Miseq Dx. Consensus sequences were classified according to PANGO Lineage and additional sublineage mutations were determined. Phylogenetic analyses were performed with Usher and MEGAX. Viral isolation was carried out on Vero/TMPRSS2 cells that were observed daily for cytopathic effect (CPE) and viral release in culture supernatant. Viral stocks are being produced for serum neutralization assays.
Results: The four patients, three fully vaccinated and one unvaccinated, aged 56-90 years. The whole-genome sequence was achieved with a genome coverage of 99.7 to 99.9 %. All sequences belonged to the PANGO lineage BA.2.3.15 and showed a unique mutation signature in the spike gene, D215E. Despite the high nucleotide identity of all sequences, a mutation in the spike gene N440K was also detected in the unvaccinated patient. The virus was promptly isolated from all patients, although it showed moderate CPE and low yield in the supernatant compared to laboratory-adapted variants.
Conclusions and discussion: WGS analysis of a cluster of SARS-CoV-2 infection in hospitalized patients demonstrated common and specific genomic mutations, confirming the epidemiological link. The Spike D215E mutation was first identified in Europe, but it is unknown whether it favours immune escape or transmission. Studies on the impact of this mutation on neutralization by monoclonal antibodies or hyperimmune plasma are ongoing. In conclusion, routine screening of patients and healthcare workers are important to restrain hospital transmission and promptly identify the emergence of new variants.

257 - Distribution and prevalence of Human papillomavirus (HPV) in oral samples collected from 2001 to 2020 in Sicily

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Distribution and prevalence of Human papillomavirus (HPV) in oral samples collected from 2001 to 2020 in Sicily

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Introduction. Human papillomaviruses (HPV) are the most common sexually transmitted pathogens. Frequently detected in the anogenital area, they can also be found in the head and neck district, where they can be associated with various conditions: from head and neck cancers (HNSCC), like oral (OSCC) and oropharyngeal squamous cell carcinomas (OPSCC) to potentially malignant oral lesions (OPMDs) such as leukoplakia (LO), oral lichen planus (LPO) and erythroplakia (EP) and benign oral papillary lesions, like condyloma acuminatum (CA) and squamous papilloma (SP). The prevalence of oral HPV positivity varies from 0% to 81% among asymptomatic individuals, and 32–36% among HNSCC patients. Data on the prevalence of oral HPV infection and its correlation with oral diseases in Italy are scarce, with the most recent study dating back to 2015 and no region-specific epidemiological analysis ever been made. Hence, the aim of this study was to depict the prevalence of Human papillomavirus infection in relation to the diagnosis of different oral ailments in Sicily.

Material and methods. The study involved a total of 1400 oral rinses consecutively received by the Virology laboratory at the Department of Hygiene and Microbiology (Policlinico University of Palermo, Italy) in the period between 2001 and 2020. The patients were referred for HPV testing after identifying oral mucosal alterations. During the twenty-year period considered, different laboratory methods were applied for the detection of HPV-DNA, depending on their availability, such as PGMY/nGP0 PCR followed by Sanger sequencing, INNO-LiPA® HPV Genotyping Extra II (Fujirebio), Roche Linear Array HPV genotyping test (Linear Array), Ampliquality HPV-Type Express v3.0 (AB analitica).

Results. HPV was detected in 11.2% of cases, of which 90.4% were single infections. HPV16 (23.3%) and HPV6 (24%) as the most common detected genotypes. Patients were mainly affected by papillomatosis (19.5%), and in 14,9% HPV was detected. OSCC represented 12.3% of diagnoses, of which 13.8% were HPV positive, while, among OMPDs leukoplakia was the most common (15,6%), with a positivity rate of 11.6%.

Discussion and conclusions. The sample cohort analyzed shows rates of HPV positivity and oral mucosal changes consistent with what has already been described in the literature. In particular, the percentage of HPV-positive OSCC, falls within the range of 1.2% to 36%, as reported in several studies carried out in Italy. The information gathered in this study will help to expand the knowledge regarding the prevalence of oral HPV in our country.

259 - Isolation of SARS-CoV-2 in viral cell culture and immunologic characterization in immunocompromised patients

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Isolation of SARS-CoV-2 in viral cell culture and immunologic characterization in immunocompromised patients

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Introduction
Immunocompromised subjects can have prolonged SARS-CoV-2shedding, even after weeks from the initial diagnosis of COVID-19.A positive molecular test does not always imply the presence of infectious virus, becausein immunocompetent individuals viral isolation declines rapidly after the first week if diagnosis.This does not appear to be the case forimmunocompromised and elderly patients who shed the virus even after weeks from infection.This study aimed at determining if SARS-CoV-2 can be isolated from immunocompromised subjects with persistently positive molecular tests and finding possible correlations with their immunological status. Finally, viral sequencing was performed to identify the variant and mutations that may favor viral persistence.

Materials and methods
The enrolled patients had a positive molecular test result for at least 21 days after their first positive test. Nasopharyngeal swabs were inoculated in Vero/TMPRSS2cells and cultures monitored daily for cytopathic effect(CPE). Virus release in supernatant wastested every three days by RT-PCR and antigenicassays. Neutralizing antibodies weretitrated by serum neutralization testperformed in microtiter plates on Vero/TMPRSS2 cells.T cell response against SARS-CoV-2 S and N proteins and CMV pp 65 proteins was evaluated by ELISPOT using Ficoll-purified peripheral blood mononuclear cells isolated from heparinized blood. Whole-genome sequencing (WGS) was performed with swabs extracted RNA and using Illumina COVIDSeq Assay kit.

Results
As expected fromprevious reports, the virus was isolated from some patients while otherswere negative. Humoral and cell-mediated responses were highly variable from individual to individual and mostly linked to their pathological conditions, drug treatment, and anti-COVID vaccine status. No clearcut correlation was found between SARS-CoV-2 isolation and respective immunological status.

Conclusions and discussion
Persistently positive molecular tests results pose serious problems for clinical management of SARS-CoV-2 patients, particularly if immunocompromised.This study can provide useful information to assess the potential risk of transmission of SARS-CoV-2 by immunocompromised individuals as related to their immunological profile. Further data are needed to identify possible correlations and determine the best strategies for patient management and prevention of transmission.

262 - Genetic diversity of norovirus Genogroups I and II in untreated wastewater in Rome Italy: a five-year monitoring study

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Introduction: Wastewater-based epidemiology applied to virology is an epidemiological complementary tool in the field of infectious disease surveillance to reveal viral circulation in the population, whether in symptomatic, asymptomatic, or preclinical states of disease. Human viruses released into feces and urine make their way into the environment by excretion or secretion of bodily fluids or skin cells. Among them, Human Norovirus (HNoV), belonging to the *Caliciviridae* viral family, is the most common etiological agent responsible of acute gastroenteritis in humans of all age. It is the leading cause of non-bacterial acute gastroenteritis (AGE) worldwide, causing approximately 18% of AGE and 212,000 deaths every year. Currently, HNoVs are divided into 10 genogroups and 49 genotypes, of which GI, GII, and GIV are known to infect humans, The aim of this study was to provide data on the genetic diversity of GI and GII HNoVs over a five-years period, testing urban sewage samples.
Materials and Methods: Between 2017 and 2021 a total of 260 sewage samples were collected from four wastewater treatment plants in Rome. Sewage concentration was performed by the two-phase polyethylene glycol (PEG), dextran separation method. Viral nucleic acids were extracted using the NucliSENS MiniMag extraction system (bioMerieux). All samples were analyzed by nested RT-PCR targeting region C (capsid gene). The PCR amplicons were pooled per year and analyzed by NGS carried out on MiSeq II sequencer (Illumina). Subsequent bioinformatic analysis was carried on with a similarity-based approach.
Results: Seven different GI capsid genotypes (GI.1, GI.2, GI.3, GI.4, GI.5, GI.6 and GI.7) were detected, and the most abundant were GI.1, GI.2 and GI.4. Among GII, thirteen genotypes (GII.2, GII.3, GII.4, GII.5, GII.6, GII.7, GII.9, GII.10, GII.12, GII.13, GII.16, GII.17 and GII.21) were reported. During the first two years, GII.2 and GII.4 were prevalent until decreasing, in 2019, for the appearance of GII.5, GII.6, GII.7 and GII.12. Across the years, GII.3, GII.13, and GII.17 were always present, and the GII.4 Sydney was the prevalent variant. This strain was first detected in Australia in 2012 and rapidly became the major epidemic strain in Europe, America and Asia in 2012–2013.
Discussion and Conclusion: Next-generation sequencing proved to be an effective strategy for HNoV genotyping in wastewater samples. A significant variety of major and minor norovirus GI and GII genotypes was reported in wastewater in Rome. Complementary data obtained from both clinical and environmental samples can be an effective strategy for understanding the diversity and evolutionary dynamics of HNoVs and infere the epidemiological status of the population.

263 - Hepatitis E in urban wastewater, pig slurry, food products, and wild boars: a One Health approach for the study of virus occurrence and diversity in the region of Abruzzo

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Hepatitis E in urban wastewater, pig slurry, food products, and wild boars: a One Health approach for the study of virus occurrence and diversity in the region of Abruzzo

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Introduction: Hepatitis E virus (HEV) infection represents an emerging public health concern worldwide. In industrialized countries, sporadic autochthonous cases are rising. Pigs and wild boars are the main animal reservoir for HEV. An ongoing project "Improving understanding of autochthonous Hepatitis E transmission routes: a focus on foodborne and waterborne pathways" focuses on the Abruzzo Region, a known hotspot for HEV circulation. Here we show the results obtained by analyzing untreated sewage samples, coastal marine water samples, raw slurry samples from swine farming activities, food samples (meat products, fruit/vegetables, bivalve shellfish), and wild boar liver samples.
Materials and Methods: Untreated sewage samples (n°= 83), coastal marine water samples (n°= 10)and raw slurry samples collected from swine farming activities (n°= 36) were collected in the Regionduring 2021. Moreover, 129 food samples (58 meat products, 45 fruit/vegetables and 26 bivalve shellfish) and 377 liver samples, taken within the control plan for wild boar population, were collectedbetween 2020 and 2021. Environmental samples were concentrated using a PEG based protocol or an adsorption/elution method. Food and liver samples were prepared with different matrix-specific protocols. A RT-nested-PCR using broad range primers targeting ORF1, and a real-time RT-qPCR were used for the screening of water and food/liver samples, respectively. Partial sequencing of the ORF2 region was performed for typing purposes, followed by analysis with the RIVM HEV typing tool.

Results: HEV RNA was detected in 10 urban sewage samples (12%) from 5 WTPs; all the samples were characterized as genotype G3, type 3c. Marine water samples were all negative for HEV. As forthe pig slurries, five samples (14%) from four different farms tested positive for HEV G3, characterized as 3f and putative subtype 3l; moreover, "unassigned" types were detected. HEV RNAwas never detected in the tested food products, while it was detected in 65/377 (17.2%) wild boar livers. Molecular characterization was achieved for 22 wild boar samples characterized as 3c, 3e, 3f,and the putative subtype 3l.

Discussion and Conclusions: HEV RNA genotype 3 was found in 12% of urban wastewaters, reflecting a not negligible viral circulation in the Region. Pig slurry was also found positive for HEV.HEV occurrence in food products, even in a hotspot region w very low. However, genotype 3 HEV is widely present in the wild boar population of Central Italy and, therefore, the use of wild boar meatand liver for food preparation should be carefully considered. An integrated Food-Animal- Environmental surveillance is fundamental for gathering data on HEV epidemiology and studying transmission routes for autochthonous hepatitis E.

265 - Antiviral effect of bombinin H2/H4 against enveloped and naked viruses

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Antiviral effect of bombinin H2/H4 against enveloped and naked viruses

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Introduction
Bombinins H (H for hydrophobic and hemolytic properties) are 20-residues amphibian antimicrobial peptides (AMP) originated from *Bombina variegata* skin secretion. These peptides are rich in glycine (25%) and can assume different conformations in addition to the typical amphiphatic α-helical structure in a membrane-mimicking environment, as shown by their circular dichroism spectra. Their most striking feature is the presence of a D-amino acid in the second N-terminal position in some of them, representing the first example of natural AMPs with a single D-amino acid in their sequence; moreover, the isomers coexist in the same secretion. In our study we focussed on Bombinin H2 and its diastereomer H4, known to exhibit high membrane-perturbing activity against Gram-negative bacteria. The aim of this study was to investigate the antiviral activity of Bombinin H2 and H4 against enveloped viruses, such as herpes simplex virus type 1 (HSV-1) and Influenza A virus (IAV), and naked virus, John Cunningham polyomavirus (JCPyV).
Methods
Vero, A549 and SVGp12 cells were infected with HSV-1, IAV and JCPyV, respectively. The efficacy of infection was evaluated by standard plaque assays and In-Cell Western assays in supernatants. Cellular monolayers were analyzed by Western blot. MTT assay was used to test possible cytotoxicity of bombinin H2 and H4 as well as to choose appropriate concentrations for further experiments.
Results
Bombinin H2 and H4 were both used at doses 10 and 20 μg/mL after testing by MTT assay. To test their antiviral effect against HSV-1, IAV and JCPyV, cells were infected and treated with H2 or H4 (10 and 20 μg/ml) at different times of infection: before (PRE), during (D) or after (P) the virus adsorption to the host cells or during and after (D+P, double dose) the adsorption phase and for the following 24/48 h. Moreover, to verify the potentially direct effect of bombinins on virions, viruses were preincubated with each peptide, and then the mixtures were used to infect the cells. We found that bombinin H2 and H4 exert virucidal effect on both enveloped viruses, but bombinin H2 is more effective than H4. No significant decrease of HSV-1 and IAV infection was observed when the peptides were used at different times of infection, nor when used to pre-treat the cells. We also found that H2 reduces JCPyV infection, probably affecting both the earliest phases of its life-cycle and the viral particle, likely through an interaction with the viral capsid proteins.
Conclusions
Overall, these data indicate that Bombinin H2 and H4 could be promising AMPs for the treatment of viral infections. This study paves the way for the potential development of short naturally occurring peptides as novel antiviral agents to enlarge the available drug portfolio.

273 - Comparative assessment of molecular methods for studying TTV-virome species composition and prevalence estimates

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Title: Comparative assessment of molecular methods for studying TTV-virome species composition and prevalence estimates

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Introduction: In recent years, interest has grown in the use of Torquetenovirus (TTV), the main component of human virome, for the prediction of post-transplant complications such as severe infections or transplant rejection. Due to the high degree of diversity, the complete genetic TTV characterization over time is poorly understood and constantly evolving. To overcome this challenge, the present study aims to investigate the individual TTV profiles in transplanted and healthy subjects by using three different molecular approaches: metagenomic next-generation sequencing (mNGS), NGS-based on TTV-amplicons (TTV-NGS), and semi-quantitative TTV species-specific PCRs (ssPCR). The final objective is to assess the complexity and diversity of TTV-virome in the study population.

Materials and methods: A total of 30 plasma samples (9liver transplanted, 10 kidney transplanted, 11 healthy) were analyzed using the three different molecular approaches. Bioinformatic analysis of the sequences was performed by setting up an in-house bioinformatics pipeline.

Results: A high degree of complexity was identified in TTV species in healthy and transplanted subjects. Both NGS methods resulted in a good performance in terms of accuracy and reproducibility, however, the TTV-NGS method showed higher sensitivity than mNGS, and was able to improve TTV classification, especially for samples with low viral load. The ssPCRs revealed a high degree of concordance with both NGS methods, although some TTV species were sub-optimally amplified. A not significant correlation was found between the number of reads associated with a TTV species and the threshold cycle expressed in the ssPCR. Finally, also the prevalence of other anelloviruses was assessed. TTMV (genus betatorquevirus) was present in the majority of patients, and TTMDV (genus gammatorquevirus) was found in a few cases only.

Conclusions and discussion: This study represents the first comparative study between different molecular approaches to characterize the TTV-virome present in solid organ transplant recipients and healthy subjects. The development of in-house methods based on real-time PCR, specific for the detection of TTV species, is an important and simple tool for investigating the dynamic viral composition of this virus, so widespread but still poorly understood in its enormous genetic variability.

276 - Wastewater surveillance of SARS-CoV-2 in Italy: outcomes of nine months of nationwide monitoring

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Wastewater surveillance of SARS-CoV-2 in Italy: outcomes of nine months of nationwide monitoring

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Introduction: Wastewater Surveillance represents an additional public health tool to investigate COVID-19 spread in a community. Following the EU Commission Recommendation 2021/472 "on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU" existing research activities were transformed into a national surveillance system, coordinated by Istituto Superiore di Sanità (ISS). Here we report the results of the environmental surveillance obtained during the period October 2021-June 2022. Results focus on the trend analysis of SARS-CoV-2 in sewage over time, as a descriptor of the dynamic of excretion of the virus in human faeces, and on the study of SARS-CoV-2 variants by sequencing of wastewater samples, performed throughout the country by regular monthly "*flash surveys*".
Materials and Methods: The SARI project (Surveillance of SARS-CoV-2 in urban sewage in Italy) for the environmental surveillance of SARS-CoV-2, implemented a national network with the participation of 20 Italian Regions and Autonomous Provinces (A.P.) as on June 2022, and 167 Wastewater Treatment Plants involved as sampling points. The laboratories for analytical activities include wastewater service providers, regional environmental protection agencies, local health authorities, zooprophylactic institutes, universities, and research institutions. Viral concentration, RNA extraction and Real time quantitative PCR (ORF1b region, nsp14) are performed by the laboratories of the SARI network using a common protocol and quantitative data are included in a GIS database implemented by ISS. For the monthly "*flash surveys*" aimed at investigating SARS-CoV-2 variants, the RNAs extracted from samples collected within one week are sent to ISS for sequencing of the spike protein, performed by both Sanger and next-generation sequencing using a MinION platform.
Results: Between October 2021-June 2022, 6694 wastewater samples were collected, and SARS-CoV-2 RNA was detected in 88% of the samples. Trends of SARS-CoV-2 concentrations in sewage, illustrated by means of Quiver graphs, show three main "waves" during the study period, in agreement with the waves observed in clinical cases. Sequencing of wastewater allowed to describe the replacement of SARS-CoV-2 variants along time, with the Delta variant predominant up to the last quarter of 2021. Since December 2021, Omicron took over in the whole country, with lineages BA.1, BA.2 and BA.5 appearing one after the other.
Discussion and Conclusions: We described trends and genetic diversity of SARS-CoV-2 in the Italian population and territory during the official environmental surveillance, along a nine-months period, confirming the effectiveness of sewage monitoring as a surveillance tool for SARS-CoV-2.

278 - Utility of genotypic CMV-drug resistance testing in immunocompromised patients

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Utility of genotypic CMV-drug resistance testing in immunocompromised patients

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Introduction

Prolonged exposure to antiviral drugs used to prevent/treat cytomegalovirus (CMV) infections in immunocompromised patients can lead to the onset of mutations in the viral genome associated to drug resistance (DR). The study evaluates the clinical utility of genotypic testing for CMV-DR in this population.

Materials and methods

Seventy-one samples from 53 patients (28 hematopoietic stem cell transplant [HSCT] recipients, 23 solid organ recipients, 2 with AIDS and 1 with autoimmune disease) with CMV-DNAemia increase/persistence after more than 2 weeks of standard-dose antiviral therapy were tested. UL97 and UL54 genes were sequenced by Sanger method to identify resistance-associated mutations (r) for ganciclovir (GCV), foscarnet (FOS), cidofovir (CDV) and maribavir (MBV); UL56, UL89 and UL51 genes to assess resistance to letermovir (LTV).

Results

CMV-DR mutations were identified in 20/53 patients (37.7%). Specifically, 13 patients (65%) treated with GCV, had single or multiple mutations in UL97 (n=6 L595S, n=5 A594V, n=2 M460V, n=1 C603R and n=1 M460I) associated with GCVr. Therapy was replaced with FOS administration in 12 cases and LTV in 1 case; only 1 patient developed CMV-related symptoms (CMV myocarditis). In 4 patients mutations on both UL97 and UL54 genes were identified and correlated with multi-DR: A594V (UL97) and P522S (UL54) associated with GCVr and CDVr, such as C603W (UL97) and P522S (UL54), A594P and H520Q (UL97) and V715M (UL54) associated with GCVr and FOSr, M460V (UL97) and Q578H and E756D (UL54) associated with GCVr, CDVr and FOSr. In these patients, immunosuppressive therapy was reduced, FOS was administered in 2 cases, MBV was administered in 1 case, and anti-CMV immunoglobulin (IgG) and leflunomide were administered in 1 case. One patient developed CMV retinitis. In 1 patient, mutations on UL97 and UL56 genes were identified in sequential samples after the exposure to each drug: first A594V (UL97) associated with GCVr, then C325Y and C325R (UL56) associated with LTVr and finally T409M and H411Y (UL97) associated with MBVr. The patient developed a CMV myocarditis. Single mutations on the UL56 gene (C325Y, R369S and C325F) associated with LTVr were detected for 3 HSCT recipients undergoing LTV treatment. For these, LTV treatment was

stopped and GCV standard dose and in one case FOS was administered. Finally, in the remaining 33/53 cases no CMV-DR mutations were detected. Among them, 2/33 (6.4%) patients developed CMV-related diseases (a chorioretinitis and renal dysfunction) and 1/33 patient died.

Discussion and conclusions

Genotypic testing for CMV-DR identifies specific mutations that guide the choice of the most appropriate antiviral therapy and limits inappropriate administration of second-line drugs.

280 - Antiviral potential of Galdieria sulphuraria, an extremophilic red alga

Antiviral potential of *Galdieria sulphuraria*, an extremophilic red alga

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Aim of the study: The recent Covid19 pandemic has shown how a single microorganism could affect the entire world, causing millions of death and job losses, with a significant psychological, social, and economic impact. As a result, the interest in alternative active compounds has increased significantly. It is known that terrestrial and aquatic macro and microorganisms are a rich source of natural bioactive compounds such as antioxidants, anti-inflammatory and immunomodulant agents, and antimicrobials which they produce in particular conditions (i.e., high/low temperatures, high pressures, light intensity, competitions). In this scenario, our study focused on exploring the biological potential of the extremophilic red alga *Galdieria sulphuraria*.

Methods: The red alga was purchased from the ACUF collection and grown in Allen medium at the same condition of the isolation site. The algal culture was collected at the exponential phase to extract its secondary metabolites, and the pellet was subjected to an organic extraction with different solvents. Cytotoxicity of the extract was evaluated by the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay on Vero cells (ATCC CCL-81). The antiviral activity was determined by plaque assay against Herpes Simplex virus type 1 and 2 (HSV-1, HSV-2) as DNA viruses, and Human coronavirus 229E (HCoV-229E) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) as RNA viruses. The plaque assay was performed under four conditions: co-treatment, virus pre-treatment, cell pre-treatment, and post-treatment. The inhibitory effect of the algal extract was confirmed by the quantitative RT-PCR, in which we analyzed the relative expression of viral genes. We explored the potential immunomodulatory activity by stimulating macrophages with 2 µg/mL of lipopolysaccharide (LPS) and then treating them with different extract concentrations. The amount of pro-inflammatory mediators and cytokines in supernatants was assessed by enzyme-linked immunosorbent assay (ELISA) kit.

The extract was subjected to a chemical characterization by the UHPLC method, carried out on a NEXERA UHPLC system (Shimadzu, Tokyo, Japan). The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both acidified with 0.1% formic acid. HR-MS analyses were performed using the AB SCIEX TripleTOF® 4600 spectrometer (AB Sciex, Concord, ON, Canada), equipped with a DuoSpray™ ion source operating in both negative and positive electrospray (ESI) ion mode.

Results: The crude extract exhibited a huge antiviral activity at the non-toxic concentrations against all the tested viruses in virus pre-treatment, particularly against the members of the Coronaviridae family, i.e., HCoV-229E and SARS-CoV-2, with an IC50 of 0.8 and 6.3 µg/mL respectively. The molecular test confirmed the data obtained in vitro. In particular, in the virus pre-treatment, no detectable levels of the genes were recorded at the highest concentrations, while reduced expression levels were found at a lower concentration. The HPLC Mass spectrometry analysis disclosed the presence of 12 compounds, mainly lipids, besides two chlorophyll derivatives. In particular, compounds eluting at 23.308 and 30.259 were tentatively identified as pheophorbide a and hydroxypheophytin a. Finally, the addition of the extract in stimulating macrophages results in a general reduction of the cytokines levels.

Discussion and Conclusions: The potent antiviral activity in virus pre-treatment against DNA and RNA viruses, whose common feature is the presence of the envelope, indicates the algal extract could act on the viral envelope inhibiting cell entry. As the UPLC analysis revealed, a possible explanation is the presence in the extract of polyunsaturated fatty acids, well-known for their antimicrobial activity due to their capability to induce the complete disruption of microbial cell

membranes and viral envelope. Moreover, it is reported that pheophorbide a disclosed a strong antiviral activity against a broad range of enveloped viruses, including SARS-CoV-2. The presence of hydroxypheophytin could explain the reported immunomodulation, an anti-inflammatory, gastroprotective, and ulcerogenic compound found in red algae. *G. sulphuraria* is considered a promising organism thanks to its extremophilic properties. Furthermore, it has been demonstrated that it has a good potential for biofuel production and bioremediation[5]. In the present study, we demonstrated its biological potential could be applied not only to the environmental fields but to the pharmacological too.

04 Batteriologia, micologia e parassitologia

2 - Climatic zone and soil properties determine the biodiversity of the soil bacterial communities associated to native plants from desert areas of North-Central Algeria

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Climatic zone and soil properties determine the biodiversity of the soil bacterial communities associated to native plants from desert areas of North-Central Algeria

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1. Introduction

Algeria is the largest Country in Africa characterized by semi-arid and arid sites, located in the North, and hypersaline zones in the Center and South of the Country. Several autochthonous plants are well known as medicinal plants, having in common tolerance to aridity, drought, and salinity. In their natural environment, they live with a great number of microbial species that altogether are indicated as plant microbiota, while the plants are now viewed as a "holobiont". In this work, the microbiota of the soil associated to the roots of fourteen economically relevant autochthonous plants from Algeria have been characterized by an innovative metagenomic approach.

2. Materials and methods

Soil samples were collected during September 2018, in two different Algerian regions (Ghardaïa and Djelfa), in six different provinces in correspondence of 14 active plant species. Four species (*C. arabica*, *R. villosa*, *Z. spinosa*, *P. undulata*) were sampled in the arid region of Ghardaïa and ten species (*A. scoparium*, *A. armatus*, *R. raetam*, *S.*

tenacissima, *A. herba-alba*, *S. tetragona*, *A. halimus*, *P. harmala*, *S. fruticosa*, *T. microphylla*) were sampled in the semi-arid region of Djelfa.

DNA was extracted from soil using the DNeasy® PowerSoil® kit. The bacterial 16S DNA libraries were prepared using the Microbiota solution B kits. The amplicon pool was processed using the Nano Kit v2 kit using Miseq platform. Raw sequences were processed by MicrobAT software. Statistical analysis was performed using both MicrobiomeAnalyst and R softwares.

3. Results

The number of observed species differed significantly according to the plant species and sampling site while Shannon's diversity index differed significantly according to the plant species, the sampling site, and the climatic zone. Seventeen bacterial species recognized as component of the core microbiota were shared between arid and semi-arid zones. Specific signature highlighted that Actinobacteria, Bacteroidetes and Proteobacteria were more represented in semi-arid zone in respect to arid one, where Actinobacteria (different genera), Proteobacteria (different genera), Chloroflexi, Planctomycetes and Firmicutes were the most prevalent. Specific bacterial signature was outlined in the different climatic zones.

4. Discussion and conclusions

The results demonstrated that in highly stressful environmental conditions, such as in desert environments, the extreme climatic conditions and the composition of the substrate are the main variables affecting the selection and recruitment of bacterial populations. In fact, specific signatures associated with the different conditions were identified for the first time, filling a gap in the current literature.

3 - Sequencing of the whole genome of Acinetobacter baumannii: monitoring of antibiotic resistance in hospital during the Sars-CoV-2 pandemic.

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Sequencing of the whole genome of *Acinetobacter baumannii*: monitoring of antibiotic resistance in hospital during the Sars-CoV-2 pandemic

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INTRODUCTION

The importance of antibiotic-resistance phenomenon and its worldwide spread have given rise to the activation of numerous surveillance systems. To make the data collected by these systems homogeneous and interpretable and to facilitate comparison between the various countries, a European surveillance network was created in 2000 which in 2010 assumed institutional characteristics becoming the European network European Antimicrobial Resistance Surveillance Network (EARS-Net) coordinated by the ECDC.

The aim of this work was to evaluate the antibiotic-resistance profile from set of 24 whole genomes of *A. baumannii* strains isolated from different departments of Alessandria (Italy) hospital, in the COVID-19 era.

MATERIALS AND METHODS

The isolation of *A. baumannii* strains took place in the period of the Sars-CoV-2 pandemic. Genomic DNA was extracted from over-night cultures in Mueller-Hinton Broth, using the DNeasy® UltraClean® Microbial kit. The DNA was then quantified, and shotgun libraries built using the Nextera XT DNA Library Prep kit. After product purification, the libraries were normalized and sequenced using an Illumina MiSeq sequencer. The obtained sequences were bioinformatically analysed using different softwares: FastQC, Trimmomatic, SPAdes, CheckM, Mash, Prodigal, EggNOG, PhyloPhlAn, and RGI.

RESULTS

Here, we propose a new experimental and computational workflow to map epidemic clusters at the hospital level using a phylogenetic analysis based on Mash Index and the Resistance Gene Identifier software to determine the genomic similarity/diversity among the isolated strains. The comparisons presented in this work highlighted the presence in our data of two strains, BAU-3 and BAU-6, different from all the others. Within this latter cluster there are further divisions, more circumscribed clusters. Specifically, the cluster containing the BAU-23, BAU-15, BAU-6, BAU-19, BAU-14, BAU-22 and BAU-11 was different from BAU-6 strain.

DISCUSSION AND CONCLUSIONS

We proposed the usage of the Mash Index, and the relative thresholds, as reliable tool in determining the similarity among strains and in monitoring their diffusion. In the framework of our data, we hypothesize that strains, except BAU-3 and BAU-6, may be the same strain because the differences between the reconstructed genomes are minimal. It should be emphasized, however, that the distance calculated with the Mash Index does not take into account the plasmid DNA, which is very important from the point of view of the mutations that are lost.

9 - Monitoring of fungal biodiversity in sea beaches of Sicily: a one-year environmental study

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Monitoring of fungal biodiversity in sea beaches of Sicily: a one-year environmental study

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INTRODUCTION

Recreational waters and tidally-wetted sands represent an eligible matrix for the development of pathogenic microorganisms. A high percentage of organic matter and relative humidity create an ideal substrate for pathogens deriving from exogenous sources closely related to human activities. Considering beach environments as a potential reservoir for pathogens, several studies showed the presence of different types of microorganisms through laboratory culture-based methods. Our study aims to demonstrate how fungal species should even be carefully monitored.

MATERIALS AND METHODS

A one-year study (2017) was performed on three recreational beaches in Sicily (Italy). Sand and water samples were collected during different seasons, registering humidity rates and weather conditions. A handful of sand was collected from three points of the same beach, while a 250 ml Schott flask was filled with water. 40 g from sand samples were diluted in 40 ml of sterile distilled water, agitated for 30 minutes (100 rpm) and plated (0.2 ml) onto Sabouraud Dextrose agar (2%) and Mycosel agar which were respectively stored at 37°C for 10 days and at 25°C for 20 days. Water samples were not diluted and directly plated under the same conditions. Identification was performed through macroscopic and microscopic observation of colonies.

RESULTS

Each beach presented equal rates of yeasts and non-dermatophytic moulds, while dermatophytes were rarely observed. The most detected fungal species were *Candida albicans*, *Geotrichum candidum*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus* and *Penicillium notatum*. *Fusarium oxysporum*, *Trichophyton tonsurans* and *Trichophyton mentagrophytes* were barely identified. Non-dermatophytic moulds were always detectable, without significant variations depending on humidity or weather conditions. Otherwise, yeast and dermatophytic isolates were mostly present during warm climate conditions (average humidity equal to 75% and temperature of approximately 26°C).

DISCUSSION AND CONCLUSION

According to our data, several fungal isolates potentially related to clinical conditions are highly represented in Southern Italy beach environments. Microbial contamination of water and sand is intimately connected, due to ordinary pollution sources and climate factors (tides, waves, wind) which promote contact between both matrices. Routinary monitoring of recreational waters is based on the presence and concentration of indicators of faecal contamination such as *Escherichia coli* and enterococci. Although these established procedures, we strongly recommend monitoring both waters and sands for fungal isolates, which could be involved in human infectious diseases.

10 - A case of rhino-pharyngeal myasis from Eastern Sicily: rare infectious diseases have their time for revenge

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A case of rhino-pharyngeal myasis from Eastern Sicily: rare infectious diseases have their time for revenge

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INTRODUCTION

Mucosal myasis is defined as a vertebrate's infectious disease caused by dipterous larvae of the *Calliphoridae*, *Oestridae*, and *Sarcophagidae* families. Animals from rural areas represent its most eligible target, even if accidental human infestations have been reported. A sheep and goat livestock parasite called *Oestrus ovis* could cause nasal myasis, characterized by nasopharyngeal symptoms. Here we describe a rare *Oestrus ovis* nasal myasis in a patient with no history of rural stay. The case aims to demonstrate how uncommon diseases could have variable distribution rates, leading to difficulties in timely recognition.

MATERIALS AND METHODS

A healthy young man came to the Emergency Room of University Hospital Policlinico in Catania, suffering from prolonged nasal itch and feeling a foreign presence in the nasopharyngeal cavity. His frequent sneezing was followed by the expulsion of white cottony material from his nostrils and mouth. Anamnestic investigation revealed a previous close contact with a dipterous during an amatorial photoshoot in a maritime area of Eastern Sicily. Physical and computerized exams were firstly required. A clinical sample spontaneously derived from the patients' nasopharyngeal cavity was analyzed at the Parasitology Unit. The University Entomology Department completed a definitive identification.

RESULTS

On physical exam, the patient's nasal mucosa was erythematous, thickened and bled easily from both nostrils. A computed tomography (CT) scan revealed mucosal oedema and sinuses free from clinical involvement. Parasitological macroscopic and microscopic analysis revealed the evidence of several larvae, that encouraged the suspicion of a myasis. These results required further investigations through specific morphological methods performed by the University Entomology Department. Larvae were identified as *Oestrus ovis* species members. The patient spontaneously resolved the infestation by expelling all the larvae, due to the lack of sinus involvement.

DISCUSSION AND CONCLUSIONS

Nasal myasis represent an uncommon and accidental event in humans. Literature reports a few cases, mostly related to stable stays in rural areas, precarious sanitary conditions, or close contact with livestock. Our case shows how rare infectious diseases could involve patients who temporally cross no rural areas due to the variable distribution of aetiological agents' habitats. Myasis from healthy patients is often sceptically considered by clinicians. This trend leads to underestimated infections and diagnostics delays. It is essential to perform a prompt and definitive diagnosis, considering rare infections as an occurrence even if typical conditions are absent.

12 - Increasing the odds of discovering novel metabolites from a large microbial library

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Increasing the odds of discovering novel metabolites from a large microbial library

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Introduction

Microbial cultures are the source of many bioactive molecules. With tens of thousands of molecules reported in the literature over the past 80 years, one of the main issues in natural product screening is avoiding the re-discovery of known metabolites. Naicons strain collection, comprising ca 45000 actinomycete strains, is a valuable source of metabolic diversity. In our screening projects we applied the use of a metabolic fingerprint library based on LCMS analysis of >14000 extracts to rapidly identify uncommon metabolites. We report here some of the novel metabolites identified through this strategy.

Materials and Methods

Actinomycete strains from the NAICONS collection were cultured in different liquid media. Culture extracts were prepared from biomass by ethanol extraction and from spent medium by solid phase extraction. Metabolites were analysed by LC-MS followed by molecular networking through GNPS platform (Global Natural Product Social Molecular Networking). Antibiotic activity of purified metabolites was evaluated using CLSI procedures. Structural characterization of compounds was based on LC-MS and NMR analyses.

Results

Extracts obtained from actinomycete strains were analysed by LC-MS and compared with an internal library containing data from >14000 extracts deriving from >4000 strains. An untargeted approach was followed, focusing on rarity and/or novelty of the signal. This approach led to several novel and interesting molecules, some of which are described herein. Biarylitides are unusual cyclic tripeptides identified in extracts from *Planomonospora* strains. Genome analysis showed that the peptides derive from 5-amino acid, ribosomally synthesized precursors, and that the corresponding minimal gene cluster is actually widespread among actinomycetes. Allopeptimicins A, produced by a member of the *Actinoallomurus* genus, are acylated cyclodepsipeptides active against Gram-positive bacteria. Conversion of the amino group in the polyketide chain to a sulfamide abolished antibacterial activity. A *Streptomyces* strain produced *N*-acetyl-cysteinylated streptophenazines, molecules that probably result from the addition of mycothiol on the streptophenazine core.

Discussion and Conclusions

Using a metabolomic approach, novel family of metabolites can be readily identified, some possessing interesting bioactivities, from unusual and common genera alike. Despite decades of intensive screening, microbial cultures continue to provide novel chemical entities when appropriate techniques are applied.

14 - Changes in microbial aetiology of Prosthetic Joint Infections: a three years evaluation.

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Changes in microbial aetiology of Prosthetic Joint Infections: a three years evaluation

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INTRODUCTION:

Periprosthetic joint infections (PJI) are the most feared complications in orthopaedic surgery, although occurring in 1-2% of primary arthroplasties, and are mainly related to biofilm-forming bacteria. PJIs are mostly caused by Gram-positive cocci, but in the last years, literature highlighted PJI cases involving Gram-negatives, including multi-drug resistant strains (MDR). PJI etiological agents can be isolated from tissue biopsies and implants. The aim is to investigate PJI aetiology in 2019, 2020 and 2021 at the Orthopedic ward of Policlinico of Catania.

MATERIALS AND METHODS:

Data from 50 orthopaedic patients with infections that underwent surgery mostly for hip (45%) or knee (20%) revision arthroplasties (figure 1) were analyzed. Overall infection percentage in orthopaedic patients was 2.2%. Removed prostheses were treated with dithiothreitol to dislodge biofilm, which was confirmed by mucosal colonies, especially for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Tissue biopsies were also analyzed.

RESULTS:

In 2019, 11 isolates were from prosthesis (including n.1 MRSA, 1 *Escherichia coli*, 1 *Proteus mirabilis*, 1 *Enterococcus faecium* strains) and 6 from tissue biopsies, among which *Pseudomonas aeruginosa*, (1) *Enterococcus faecalis* (2) and MRSA (2). In 2020, from 8 tissue biopsies, we isolated 1 MRSA, 1 *E. faecalis*, 2 *P. mirabilis*, 1 *Acinetobacter baumannii*, while from prosthesis cultures we obtained 3 *S. aureus*, 2 *A. baumannii*, 1 *E. faecium*, 4 *Enterobacterales*, 1 *P. aeruginosa*. In 2021, from 7 implants we isolated 1 *S. aureus*, 1 VRE, 1 KPC, 1 *A. baumannii* and from 5 tissue biopsies we isolated 1 *A. baumannii*, 2 *S. aureus*, 2 *P. aeruginosa*. Considering susceptibility, most of the Gram-negatives were resistant to carbapenems (KPC positive), 3dG cephalosporins and Fqs but susceptible to protected cephalosporins.

DISCUSSION AND CONCLUSIONS:

Data from this 3-year study in which a percentage of infection almost similar was observed, documented a relevant change occurred in bacteria involved in PJIs. In 2019, mostly Gram-positive cocci have been isolated, with an important prevalence of *S. aureus*. In 2020, Gram-positives (mostly *S. aureus*) and Gram-negatives, including MDR bacteria, were present equally. In 2021, Gram-negatives were more represented, with a significant prevalence of MDR microorganisms. Further analysis including biofilm formation and new antibiotic treatments are ongoing.

16 - Interference on uterine bacterial population in hypofertile mares of intrauterine infusion of equine fresh platelets-rich plasma.

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Interference on uterine bacterial population in hypofertile mares of intrauterine infusion of equine fresh platelets-rich plasma.

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Introduction The clinical use of platelet-rich plasma (PRP) in equine is increasing. Several *in vivo* and *in vitro* experiments have shown beneficial effects of PRP in modulation of the inflammatory process and tissue healing. PRP is an emerging therapeutic application also for persistent breeding-induced endometritis, which is one of the major causes of infertility in mares. This study aims to investigate whether intrauterine PRP infusion interferes with the uterine microbial population of hypofertile mares. **Materials and Methods** In the year 2022, low-volume uterine flush (150 ml) was collected from 12 in estrus mares presenting fertility problems (barren in preceding season or repeated breeder), before and after about 12 h the PRP intrauterine infusion treatment. All mares were artificially inseminated 10 h before the first lavage. The samples were processed at the Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production, University of Naples "Federico II" (Italy). They were inoculated in the broth-enrichment Brain Heart Infusion (BHI) and incubated aerobically at 37°C for 24 h. The day after, turbid BHI tubes were sub-cultured on different types of solid culture agar media and incubated aerobically at 37°C for 24-48 h. The isolates were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). **Results** We observed that the uterine flush samples from three Italian trotter mares resulted to be negative for bacterial growth, whereas the same bacterial species were detected from four mares both before and after PRP treatment. Moreover, after PRP treatment an increase of the bacterial species in four mares was observed, while in a single mare we detected *E. coli* growth before treatment but no bacterial growth after PRP treatment. The most isolated bacterial species was *Streptococcus equi* subspecies *zooepidemicus*, which was detected both before and after PRP treatment except in a mare, which resulted to be negative before and positive to *Streptococcus equi* subspecies *zooepidemicus* and *Klebsiella pneumoniae* after the PRP treatment. **Discussion and Conclusions** Although PRP is found to have antimicrobial properties and has been widely used in veterinary medicine, no study is known so far on the interference of PRP intrauterine infusion treatment on uterine bacterial population in hypofertile mares. This preliminary study demonstrated that PRP intrauterine infusion treatment appeared not to interfere with the viable uterine microbial community composition.

18 - The spread of fluconazole-resistant Candida parapsilosis strains during the global COVID-19 pandemic in a Northern Italy hospital.

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The spread of fluconazole-resistant Candida parapsilosis strains during the global COVID-19 pandemic in a Northern Italy hospital.

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Introduction: The global pandemic of COVID-19 has predisposed a relatively high number of patients to acute respiratory distress syndrome, which carries a risk to develop super-infections. Candida parapsilosis (CPA), the most common species of Candida non albicans isolated from bloodstream infections, although usually susceptible to azoles, is has increased significantly in recent years and it has been reported as an emerging multi-resistant yeast. The current SARS-CoV-2 pandemic has evidenced that COVID-19 patients are more susceptible to secondary infections. The aim of this work was to evaluate the prevalence of fluconazole resistant-CPA (CPA-R) in our hospital during SARS-CoV-2 pandemic period.

Materials and Methods: This study, conducted at the "Guglielmo da Saliceto" Hospital (Piacenza), refers to the period January 2019 - March 2022. CPA isolates from blood cultures were divided according to both isolation period and susceptibility/resistance to azole; period 1 (January 2019 - March 2020; control period) and period 2 (January 2021-March 2022; SARS-CoV-2 pandemic period).Yeast identification was performed by using MALDI-TOF MS (Vitek MS, bioMérieux, France) and antifungal susceptibility was evaluated by using the Vitek2 system (bioMériueux, France) and confirmed by Sensititre Yeast One YO10 (Thermofisher Scientific,USA) microdilution broth. ERG11 gene of 24/32 CPA fluconazole-resistant strains isolated from January 2021 to March 2022 was analyzed by sequencing according to Sanger method. Statistical analysis was performed according to Fisher Exact Test.

Results: As shown in Table 1, CPA isolates had a significant increase of +135,7 % ($p<0.01$) in Period 2 compared to Period 1. Moreover, we observed increase of CPA-R strains in the same period, according to the data shown in Table 1. The evaluation of ERG11 gene sequences in 24 of 32 strains in Period 2 allowed to highlight the presence of Y132F mutation in almost all CPA-R strains (23/24). The detection of R398I mutation is detected in only one CPA-R (1/24).

	Period 1	Period 2
Total CPA	28	66
CPA-S (%)	22 (78,6%)	34 (51,5%)
CPA-R (%)	6 (21,4%)	32 (48,5%)

Table 1. Numbers and percentages of Candida parapsilosis in blood infections, and in particular, CPA fluconazole susceptible (CPA-S) strains and CPA-R strains in the two periods of the study.

Discussion and Conclusions: In this study we observed a substantial increase of CPA bloodstream infections during period 2, especially the ones caused by CPA-R strains. This could be explained by the reduction of infection control practices during a pandemic period and subsequent spreading of multi-resistant pathogens. The presence of the same mutation in the azole-resistant strains suggests the possible existence of a spreading cluster. Further investigations will be performed through NGS sequencing to confirm our suspicion.

20 - Acinetobacter baumannii and cefiderocol between cidality and adaptability

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Acinetobacter baumannii **and cefiderocol between** ***cidality*** **and adaptability**

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Introduction: Among the bacterial species included in the ESKAPE group, *Acinetobacter baumannii* is of great interest due to its intrinsic and acquired resistance to many antibiotics and its ability to infect different body districts. Cefiderocol (FDC) is a novel cephalosporin active against Gram-negative bacteria with promising efficacy on *A. baumannii* infections, but some studies have reported therapeutic failures even in the presence of susceptible strains.

Materials and Methods: This study aims to investigate the interactions between FDC and ten *A. baumannii* strains through determination of their susceptibility to FDC using both disk diffusion and broth microdilution as well as MBC/MIC evaluation to determine the bactericidal effect of FDC. Heteroresistance and adaptation of *A. baumannii* to FDC were evaluated by population analysis profile and induction assay respectively. Genomic analyses were performed to assess the presence of mutations related to the different FDC resistance profile. Eventually, the efficacy of beta-lactamase inhibitors (BLIs) in restoring the activity of FDC was evaluated.

Results: We confirmed diverse susceptibility profiles, with resistance values close to the EUCAST-proposed breakpoints in some strains. MBC/MIC demonstrated bactericidal activity of the drug with ratio values ≤4 for all the strains but ATCC 19606; on the other hand, bacterial regrowth was evident after exposition to FDC, as were changes in the shape of colonies and bacterial cells. A switch to a non-susceptible phenotype in the presence of high FDC concentrations was found in one strain as adaptation mechanism implemented to overcome the *cidal* activity of this antibiotic, also confirmed by the presence of heteroresistant, unstable subpopulations in 8/10 samples. Genomic analyses revealed the presence of mutations in PBP3 and TonB shared by all the strains regardless to their resistance phenotype. BLIs showed the ability to restore the antimicrobial activity of FDC despite the different non-susceptibility levels of the tested strains.

Discussion and Conclusions: By investigating the resistance profile of some clinical and laboratory-adapted *A. baumannii* strains to FDC, we found that these strains are not easily classified into the commonly used categories of susceptibility - especially strains with susceptibility levels very close to the proposed resistance breakpoints – due to a high prevalence of heteroresistant subpopulations. Heteroresistance in *A. baumannii* seems to be common as a stress response mechanism, but - luckily - it is apparently an unstable and transient trait. These *in vitro* results, can sustain the concept of using combination therapy to eliminate drug-adapted subpopulations and regain full FDC activity in this difficult-to-treat species.

22 - Combination of essential oils to poly(ε-caprolactone)-based biomaterial to achieve anti-bacterial and osteoblast proliferative properties for regenerative medicine scaffolds.

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Combination of essential oils to poly(ε-caprolactone)-based biomaterial to achieve anti-bacterial and osteoblast proliferative properties for regenerative medicine scaffolds.

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Introduction: Biomedical implants, an essential part of the medical treatments, still suffer from bacterial infections that hamper patients' recovery and lives. Antibiotics are widely used to treat those infections but brought to antibiotic resistance. Essential oils (EOs), a complex mixture of different natural biomolecules, demonstrate excellent antimicrobial activity, being active against fungi and a wide range of gram-negative and gram-positive bacteria, and low resistance development risk. However, EOs application in medicine is still quite scarce and almost no research work considers its use in combination with bioresorbable biomaterials, such as poly (ε-caprolactone) (PCL) polymer. This work aimed to combine the antibacterial properties of EOs, particularly eugenol and cinnamon, with those of PCL for medical applications in which good tissue regeneration and antimicrobial effects are required.
Materials and methods: PCL porous samples were developed by solvent casting combined with a salt-leaching method, in which sodium chloride and sodium nitrate were used as pore former. Different concentrations of EOs (from 30% to 50%) with respect to the PCL mass were added to the polymer. Samples were further characterized from the morphological and microstructural point of view by Field Emission Scanning Electron Microscopy (FESEM). *In vitro* direct-contact tests on sarcoma osteogenic-2 (Saos-2) cells vitality/proliferation at different incubation times were performed. Antibacterial properties of different PCL samples against *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 35984 and *Escherichia coli* ATCC 25922 were determined by both inhibition halo assay on agar and by adhesion tests that consisted in the count of adherent or planktonic bacteria.
Results: FESEM showed that the samples were characterized by square-shaped macropores, whose average dimension well matched that of the starting salt. Saos-2 cells cell vitality and proliferation was hampered by 40 and 50% EOs-enriched PCL, whereas no cytotoxic effect was recoded for both 30% EOs-added PCL and pure-PCL used as control, within 12 hours of incubation. The antibacterial tests revealed the presence of a small inhibition halo around the 30% eugenol and cinnamon-functionalized PCL scaffolds only for *S. aureus* and *S. epidermidis*, on the contrary no inhibition halo was observed for *E. coli*. A significant ($p<0.001$) 2 log decrease on adherent bacteria was achieved for all the three bacteria: from 10^9 to 10^7 CFU/mL for *S. aureus* and *E. coli*, and from 10^7 to 10^5 CFU/mL for *S. epidermidis*, independently to the EO, eugenol or cinnamon, or the porous agent used. In addition, a 1 log reduction ($p<0.05$) in the number of planktonic microorganisms was also obtained, thus proving that even if the EOs are only in part released by the EOs-added PCL scaffolds an antibacterial feature is anyway achieved.
Discussion and Conclusions: A biocompatible synthetic polymer scaffold for prosthetic implants that could enhance osteogenesis, and simultaneously prevent post-surgical infection and inflammation, is urgently needed. This research shows the great potential of EOs use for biomaterial functionalization with enhanced anti-bacterial properties without affecting osteoblasts vitality/proliferation.

23 - New drugs, new diagnostic techniques and new algorithms in fast track workflow of Gram-negative bloodstream infections: a three-year single-centre study

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New drugs, new diagnostic techniques and new algorithms in fast track workflow of Gram-negative bloodstream infections: a three-year single-centre study

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Introduction The contribution of rapid diagnostics in tackling antimicrobial resistance and improving the outcomes of patients with BSI is a matter of debate but might be enhanced by the availability of new approved drugs. In this study we evaluated a rapid diagnostic algorithm based on direct MALDI-TOF MS, lateral flow immunoassays (LFIAs) and molecular testing performed directly from positive blood cultures (BCs) for Gram-negative species identification and detection of CTX-M extended-spectrum-β-lactamases and main carbapenemases. Secondly, we evaluated the association between detected resistance markers and antimicrobial susceptibility towards main antimicrobials including those newly approved such as ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam and cefiderocol.
Methods: Non-duplicate BCs positive to Gram-negative bacteria at microscope examination were subjected to species identification by direct MALDI-TOF MS following recovery of bacterial pellet by Rapid MBT Sepsityper® kit. Subsequently, NG-Test® CARBA 5 and NG-Test® CTX-M MULTI LFIAs were performed according to identified microbial species. Eazyplex® SuperBug CRE molecular assay was performed in cases of NG-Test® CARBA 5 negative results in patients with documented carbapenemase-producers carriage. Results of rapid diagnostic workflow were compared with those obtained by conventional diagnostic routine. Associations between detected resistance markers and antimicrobial susceptibility to main antimicrobials were analysed using X^2 test.
Results: Overall, the direct MALDI-TOF MS protocol allowed reliable identification to the species level of 92% of the 2398 monomicrobial BCs. Rate of matched identification was significantly higher for Enterobacterales (97%) in comparison to non-fermenting Gram-negative species (82%), obligate anaerobic bacteria (42%) and fastidious Gram-negative species (41%). The overall sensitivity of NG-Test® CARBA 5 and NG-Test® CTX-M MULTI was 92.2% and 91.6%, respectively. Integration of Easyplex® SuperBug CRE allowed detecting bla_{KPC} mutants associated with ceftazidime/avibactam resistance, reaching 100% sensitivity in carbapenemase detection. Both LFIAs and molecular testing showed no false positive results. Statistical significant associations between detected resistance markers and susceptibility to old e new antimicrobials were observed.
Conclusion: Algorithms based on MALDI-TOF MS, LFIAs and molecular testing may represent a cost effective tool to timely identify Gram-negative species and detect resistance markers directly from BCs. According to local epidemiology, these results may allow antimicrobial stewardship interventions including prompt use of new approved drugs.

24 - Antimicrobial activity of Curcumin and Curcumin/Chitosan loaded nanobubbles with photodynamic light

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Antimicrobial activity of Curcumin and Curcumin/Chitosan loaded nanobubbles with photodynamic light

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Introduction: Curcumin is a natural substance extracted from turmeric that has *in vitro* antimicrobial properties against a wide variety of microorganisms. Moreover, due to its ability to emit fluorescence, its antimicrobial efficacy can be enhanced by photoactivation. However, the potential of this natural extract is limited by poor water solubility and instability at physiological pH, leading to poor absorption and rapid degradation by hydrolysis and molecular fragmentation. To overcome these issues, curcumin can be encapsulated in biocompatible nanosystems, which also have antibacterial properties, increasing its bioavailability and solubility. The present work aimed at investigating antimicrobial properties on both gram negative and gram positive bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) by using curcumin and curcumin/chitosan nanobubbles (NBs) with and without photodynamic treatment.

Materials and Methods: The effectiveness of curcumin alone, curcumin-chitosan loaded NBs and oxygen-curcumin-chitosan loaded NBs with and without irradiation (photodynamic blue light at 450nm) against above-mentioned bacterial strains were determined by using micro plate dilution assay for Minimum Inhibition Concentration (MIC) determination and colony counting method for Minimum Bactericidal Concentration (MBC) and time kill curves. In addition, the effect of curcumin and NBs on bacterial membrane integrity was assessed by measuring the release of lactate dehydrogenase (LDH) enzyme.

Results: We ascertained that curcumin (alone) with blue light have a robust effect against the mentioned bacterial strains. We find out MIC at a very low concentration of curcumin (0.125mg/ml, 0.0075mg/ml and 0.0037mg/ml) against *E. coli*, *S. aureus* and *E. faecalis* respectively, after three hours of LED irradiation. On the other hand, NBs showed a high antibacterial activity with MIC ranges of 0.011-0.46 mg/ml. Curcumin and NB MBC values were 1-2 times higher than MIC. Moreover, time kill kinetics confirmed that NBs were very effective to inhibit bacterial growth more than 72 hours. Bacterial membrane integrity was also checked and the results of LDH showed that NBs damaged microbial cell membranes.

Discussion and conclusions: In conclusion, it is interesting to speculate on a potential role of curcumin and curcumin loaded NBs, alone or together with photodynamic light, as to enhance antimicrobial activity. The findings of the proposed research will be helpful in fighting against harmful microorganisms.

25 - optrA-mediated linezolid resistance in an Enterococcus faecalis isolate recovered from a wild raptor (Falco peregrinus peregrinus), central Italy

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optrA-mediated linezolid resistance in an _Enterococcus faecalis_ isolate recovered from a wild raptor (_Falco peregrinus peregrinus_), central Italy

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Introduction: Enterococcus species are commensal bacteria that colonized the intestinal tract of humans, mammals, and birds, and are commonly present in the environment. *Enterococcus faecalis* have been increasingly identified as important animal and human pathogens. The emergence of linezolid-resistant enterococci (LRE) has been described in several countries and LRE isolates harbouring a *cfr* (encoding a 23S rRNA methylase) and *optrA*- and *poxtA* (encoding ABC-F ribosomal protection proteins)-containing plasmid were reported from animals, mostly from Asian countries and livestock animals. In this work, we studied an *E. faecalis* strain from a hawk and harbouring the *optrA* gene.

Materials and Methods: *E. faecalis* 30488 was isolated in June 2021 in Umbria region from an adult female Peregrine falcon (*Falco peregrinus* subspecies *peregrinus*) that died of septicemia, confirmed by culture and histopathological examination, as a probable consequence of a gunshot wound. The strain was screened for the presence of *cfr*/*cfr*-like, *optrA* and *poxtA* genes by PCR assays. Antimicrobial susceptibility testing was carried out by broth microdilution. S1-PFGE, southern blotting and hybridization with *optr*A-specific probe have been used to determine gene location. Transferability was assessed by conjugation experiments using *E. faecalis* JH2-2 as recipient. The *optrA* genetic context and clonal lineage were analyzed by Whole Genome Sequencing (WGS).

Results: *E. faecalis* 30488 harbored the *optr*A gene and exhibited resistance to linezolid, chloramphenicol, florfenicol, tetracycline and erythromycin and susceptibility to tedizolid and vancomycin. Conjugation experiments failed to demonstrate the transferability of the *optr*A gene to *E. faecalis* JH2-2. S1-PFGE and hybridization assays displayed a chromosomal localization of the *optrA* gene. Further genome analysis showed that the *optrA* gene – linked to *fexA* and adjacent to a complete Tn*554* element containing *erm*(A) and *spc* genes – was carried by a chromosomal Tn*6674*-like transposon inserted in *radC*. The Tn*6674*-like was 99% identical to the Tn*6674* first reported in a porcine *E. faecalis* strain. WGS analysis showed that the strain belonged to the ST476, normally associated with human and animal enterococci. From phylogenetic analysis the *E. faecalis* 30488 isolate was closely related to the *E. faecalis* S338 strain previously isolated from swine farms in central Italy.

Discussion and Conclusions: This is the first report of a linezolid-resistant *E. faecalis* isolate of wild raptor origin carrying *optr*A gene. The identification of acquired mechanism of resistance to linezolid in wild animals, especially in birds, is highly worrisome for the potential role of wild animals as possible reservoir of LRE among different ecological niches.

26 - Detection of an Enterococcus faecium carrying the phenicol-oxazolidinone resistance gene poxtA recovered from a freshwater river, central Italy

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Detection of an *Enterococcus faecium* carrying the phenicol-oxazolidinone resistance gene *poxtA* recovered from a freshwater river, central Italy

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Introduction: Enterococci are members of gut microbiota of human and animals. Although regarded as commensals, they are an increasing cause of nosocomial and antibiotic-resistant infections worldwide. The treatment of severe infections due to VRE and MDR enterococci is often limited to the last-resort antibiotics such as linezolid (LZD) and tedizolid (TDZ). Due to their abundance in animal feces and persistence in the environment, enterococci spread in many habitats including soil, water, food of animal origin, sewage and plants. Here we report the occurrence of an *E. faecium* isolate carrying the LZD resistance gene *poxtA* from a freshwater river.
Materials and methods: Florfenicol-resistant enterococci from water samples from the Salinello river estuary, were tested by PCR for the presence of LZD resistance genes and for their susceptibility to several antibiotics by standard broth microdilution assay. S1-PFGE and southern blotting/hybridization assays were performed to determine the gene location. Conjugative transfer was assessed both *in vitro* filter mating experiment and in aquaria microcosm assays using *E. faecium* 64/3 and *E. faecium* Ef1 as recipients. WGS was carried out using the Illumina and Nanopore platforms. The presence of circular forms was examined by inverse PCR. Plasmid stability was also assessed.
Results: *E. faecium* M1, was positive for the presence of *poxtA* gene that displayed both a chromosomal and plasmid localization. WGS analysis indicated that *poxtA* was on a 30,877-bp plasmid (pEfM1) with high identity and synteny to the enterococcal 27-kb plasmid pEfm-Ef3. In both plasmids the *poxtA* genetic context, flanked by two IS*1216*, was located in a Tn*6657*-like transposon also containing *fexB* gene; *tet*(L) and *tet*(M) genes in tandem, were located upstream of the transposon. The comparative analysis of the two plasmids showed that in pEfM1 a second copy of *poxtA* genetic context was inserted within the Tn*6657*-like upstream of the *orf8*.
The *poxtA* environment was proved to be unstable. In filter mating experiments, *poxtA* was successfully transferred from *E. faecium* M1 to both enterococcal recipients, while in aquaria microcosm assays the gene was transferred only to *E. faecium* 64/3. To test the pEfM1 stability, *E. faecium* M1 and transconjugants were subjected to serial passages in antibiotic-free plates for 30 days at 37°C and 42°C. After only three passages, both at 37°C and 42°C, the transconjugants obtained in aquaria microcosm experiments had lost the *poxtA*, but not pEfM1.
Discussion and conclusions: To our knowledge, this is the first report on the occurrence of a *poxtA*-carrying enterococcus from freshwater, in Italy. Our findings suggest that also the freshwater ecosystem could represent an important reservoir of LZD resistance genes.

35 - Evaluation of the MALDI-TOF mass spectrometry workflow for the identification of microorganisms involved in monomicrobial and polimicrobial bloodstream infections.

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Evaluation of the MALDI-TOF mass spectrometry workflow for the identification of microorganisms involved in monomicrobial and polimicrobial bloodstream infections.

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Introduction. The rapid identification (ID) of microorganisms from blood samples is critical for a prompt and adequate patient's management. The diagnosis of bloodstream infections (BSIs) traditionally relies on blood cultures (BCs) but suffer from long time-to-result. Since MALDI-TOF mass spectrometry performant in timely identifying microorganisms cultured on solid media, several in-house-pre-treatment protocols have been developed for the direct ID of microorganisms in BCs. This study aims to evaluate the MALDI-TOF Sepsityper™ workflow (MSW), focusing on mixed BSIs.

Materials and Methods. A total of 361 positive BCs was selected to perform the Sepsityper™ sample preparation procedure according to the manufacturers' instructions (Bruker, Germany). One ml of each BC was purified to obtain a pellet by a quick-to-perform purification protocol. In parallel, further aliquots of each BC were used for both microscopic examination with Gram staining technique and subculture onto solid media (CC). The detection limit of the MSW was assessed by analysing experimentally seeded monomicrobial BCs using different microorganisms. To evaluate MSW's performance in identifying mixed BSIs, 10 experimentally seeded mixtures of two microorganisms (among 7 distinct bacteria) at different concentrations for a total of 50 combinations were used.

Results. As a result of microscopic examination, the 322 monomicrobial positive BCs contained Gram-positive (GP) and Gram-negative (GN) bacteria in 64.6% (208/322) and 35.4% (114/322), respectively. An ID at species level was obtained for 89.4% (186/208) of BCs-containing-GP bacteria and for 97.4% (111/114) BCs-containing-GN bacteria. The subculture results showed 92% (296/322) agreement with the MSW results. With regard to the 39 polymicrobial BCs, the MSW gave an ID at species level, in agreement with CC, for both microorganisms in 2.6% (1/39), an ID for only one of the microorganisms in 89.7% (35/39), and no ID in 7.7% (3/39). The detection limit was assessed to range from 2.5×10^6 to 6.8×10^6 cfu/ml. As concern the experimentally seeded mixed BCs, the ID of both microorganisms was obtained for all mixtures at least in one combination.

Discussion and Conclusions. The MSW demonstrated to be a helpful tool in identifying the microorganisms from BCs. This method showed a high identification capability for both GP and GN bacteria and no misidentification was observed. With regard to mixed BSIs, more often only one of the microorganisms involved was identified at species level. In addition to its significant performance, MSW relies on a cheap, easy- and rapid-to-perform protocol highly standardized and, therefore, it demonstrated to be dependent from pre-analytic and analytic variables.

36 - Schistosoma haematobium, an imported parasite: a 5-year epidemiological study.

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***Schistosoma haematobium*, an imported parasite: a 5-year epidemiological study.**

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Introduction. Schistosomiasis is a parasitic disease caused by *Schistosoma* species pathogenic to humans. This parasitosis has long been considered a phenomenon confined to Tropics, however it is estimated than 220 million people in 78 countries are affected worldwide. The majority of the cases are associated to *S. haematobium*, the aetiological agent of urogenital schistosomiasis. Schistosomiasis is one of the most imported parasitoses in our area, therefore this study aims to evaluate the trend of the prevalence rates during a five-year period.
Materials and Methods. From 2017 to 2021, 883 cases of suspected urogenital schistosomiasis were investigated. Single and multiple urine and serum samples of the same patient were considered as a single case based on the following criteria: (i) all the samples of the same patient analysed for 1 month; (ii) the samples of a patient being always positive for *S. haematobium* continuously over a long period. In 381 and 209 cases, only serum or urine samples were analysed, respectively; for the remaining 293 cases both samples were tested. All serum samples were analysed by Schistomiasis Serology Microwell ELISA (SCIMEDX, USA). All urine samples were submitted to microscopic examination to detect *S. haematobium* ova after concentration by using a Cellulose Nitrate Microporus membrane (Thermo Scientific, USA)
Results. A total of 115 positive cases (39.3%) were revealed among the 293 cases for which both urine and serum samples were tested: in 97 cases only serum and in 1 case only urine were positive, whereas in 17 cases both samples were positive. *S. haematobium* ova were detected in 15 cases (7.2%) of the 209 for which only urine samples were tested, while a positive result was obtained in 140 cases (36.7%) of the 381 for which only serum samples were tested. Among the overall 270 positive cases, for 12.3% (33/270) *S. haematobium* ova were detected, with a decreasing trend through the years (2017: 5.3%; 2018: 4.5%; 2019: 3.7%; 2020:1.7%; 2021:1.8%).
Discussion and Conclusions. During the last two years, a decreased number of samples for urogenital schistosomiasis suspicion has been observed as well as its prevalence, probably due to the reduced migratory flows all worldwide because of the COVID-19 pandemic. Although its prevalence rate is apparently reduced in 2020 as compared to the previous years, any reliable conclusion could be affected by the low number of cases reported during the pandemic period. Overall, 270 positive cases were observed, but only in 33 cases the outcome was referred to *S. haematobium*, since the ova were detected. The high number of positive serum samples without ova detection referred to an equally number of subjects exposed to the parasite, but not infested at the time of the investigation.

38 - In vitro effect of innovative light with photodynamic microbicidal activity in bacteria

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In vitro effect of innovative light with photodynamic microbicidal activity in bacteria.

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Introduction Light energy emitted in the visible light spectrum (VIS) is a non-harmful form of radiance and can result in photodynamic microbicidal effect in bacteria. Photosensitizing chromophores, like porphyrins, are present in bacteria and are sensitive to light in the visible spectrum, particularly in the visible blue-violet spectrum (400-420 nm). These wavelengths result in excitation of porphyrins and subsequent production of singlet oxygen ($^1O_2$) and other reactive species (RS). These products damage cytoplasmic and membrane structures, causing reduction of survival, even in multidrug-resistant microorganisms. The *Biovitae* ® light, used in this study, emits white light from a light-emitting diode (LED) system but no UV spectrum. This technology has an microbicidal power on bacterial strains and viruses, without harming mammalian cells. **Materials and Methods** The tested light was provided by the *Nextense* company, and it uses a special combination of frequencies which cover the visible spectrum and create a multispectral interfering wave system for microbial eradication (MIME): 400-420 nm, 400-450 nm, 400-700 nm at an intensity of 3,51 mW/cm^2, 5,85 mW/cm^2, 12,53 mW/cm^2, respectively. The tests (n=16) were performed *in vitro* using 96-well plates and *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) strains, previously cultured in LB medium for 24 h at 37°C. We obtained an average optical density (OD_{595nm}) for *E. coli* of 0.140 A (1.1 x 10^8 cells/ml) and for *S. aureus* of 0.150 A (1.2 x 10^8 cells/ml) using Victor spectrophotometer (PerkinElmer). The absorbance of the empty culture medium was subtracted from the bacteria containing samples. The bacterial stock solutions were also diluted 1:2 and 1:4. Plates with bacteria were exposed to antimicrobial light for six hours, and bacterial growth monitored over 24 hours. **Results** During light exposure, a slight and steady increase in growth was observed for both *E. coli* and *S. aureus*. However, 18 hours after light exposure, bacterial growth significantly ($P \leq 0{,}005$) decreased compared to probes not exposed to the light. Comparison of the corresponding samples with Student's *t*-Test showed that growth reduction appears to be higher significant in the 1:2 dilution of both bacteria strains. **Discussion and conclusion** These results confirm the antimicrobial action of Biovitae ® light both against Gram-negative and Gram-positive bacteria. Although during light exposure bacterial growth seems to be stimulated, the post light growth significantly ($P \leq 0{,}005$) decreased the bacterial survival.

40 - Temporal correlation between SARS-CoV-2 infection and Candida parapsilosis bloodstream infections.

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Temporal correlation between SARS-CoV-2 infection and Candida parapsilosis bloodstream infections.

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Introduction: Candida species are major constituents of the human mycobiome and the main cause of invasive fungal infections with a high mortality rate. Invasive yeast infections are increasingly recognized as complication of severe COVID-19. Candida parapsilosis (CPA), the most common species of Candida non albicans isolated from bloodstream infections, although usually susceptible to azoles, is recently reported as an emerging multi-resistant yeast. Current SARS-CoV-2 pandemic has evidenced COVID-19 patients' susceptible to secondary fungal infections, either mould and yeast infections. Aim of this study was to investigate whether SARS-CoV-2 infection predisposes to the onset of CPA-borne candidemia.

Material and methods: This study was conducted at the "Guglielmo da Saliceto" Hospital (Piacenza) from January 2021 to March 2022. CPAs isolated from blood cultures were analyzed. Virtuo (bioMerieux) automated blood culture system was used for monitoring blood culture bottles. Yeasts were identified at species level by using VitekMS (bioMerieux) device and MALDI-TOF MS method. For patients with CPA candidemia we investigated prior SARS-CoV-2 positivity. The diagnosis of COVID-19 was made with polymerase chain reaction (PCR) for SARS-CoV-2 with RT-qPCR Alinity m SARS-CoV-2 assay (Abbott Laboratories, Chicago, IL, USA).

Results: Over the period considered, 66 patients with candidemia by CPA have been identified. Thirty-two of them (48.5%) have never tested positive for SARS-CoV-2, while the remaining 34 (51.5%) were hospitalized for COVID-19. In the latter group, time of onset of candidemia was related to time of SARS CoV-2 diagnosis: 10 (29.4%) patients contracted SARS-CoV-2 within 15 days prior to the diagnosis of candidemia, 9 (26.5%) tested positive between the 16th and 30th day, and, eventually, 15 (44.1%) contracted COVID-19 more than one month before the CPA candidemia.

Discussion and Conclusions: The collected data did not show a significant difference between CPA candidemia in SARS-CoV-2 positive and negative patients. While, analysing the three different groups of patients with SARS-CoV-2 infection, 70% of patients presented candidemia after 15 days of COVID-19 infection, and 44% after more than 30 days. The monocentric evaluation of the study and the relatively low number of analysed patients does not allow to reach statistical significance of the data, but an evident correlation between length of stay and onset of candidemia is appreciable. Clinical features and risk factors such as ICU admission and use of corticosteroids could be investigated. Moreover, further multicentric studies on specific SARS-CoV-2 predisposing factors for candida systemic infections are advisable to clarify specific pathophysiological pathway of host/viral and yeast pathogen interaction.

41 - Sexually transmitted pathogens in a selected population: a preliminary study

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Sexually transmitted pathogens in a selected population: a preliminary study

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Background

Sexually transmitted infections (STIs) are frequently diagnosed among men who have sex with men (MSM), above all in HIV positive patients. The aim of the study was to evaluate pathogens etiologically related to STIs in MSM population attending the clinic dedicated to sexually transmitted diseases (STDs) at the Azienda Ospedaliero Universitaria Policlinico, Bari, Italy.

Materials and methods

From November 2021 to May 2022, 161 samples (50 anal and 53 buccal swabs, 58 urines) collected from 56 patients, in particular 28 HIV+, 19 HIV- and 9 HIV- in pre-exposure prophylaxis (PrEP). were analyzed. In order to detect the following pathogens: Chlamydia trachomatis (Ct), Mycoplasma genitalium (Mg) and Mycoplasma hominis (Mh), Ureaplasma urealyticum (Uu) and Ureaplasma parvum (Up), Neisseria gonorrheae (Ng) and Trichomonas vaginalis (Tv), a multiplex Real-Time PCR (mRT-PCR) (AnyplexTM II STI-7, Seegene, Inc., Seoul, Korea) assay was performed.

Results

34 of 56 patients (60%), were positive for at least one of the detected microorganisms. Positivity for STIs was revealed in 20/28 (71.43%) HIV+, 10/19 (52.53%) HIV- and 4/9 (44.44%) HIV- using PrEP. We also identified and compared the most frequent sexually transmitted pathogens between HIV+, HIV- and PrEP, respectively. The results were as: Uu (12.35% in HIV+, 14.81% in HIV- vs 11.32% in PrEP), Mh (9.88% in HIV+, 7.55% in HIV- vs 3.70% in PrEP), Mg (3.70% in HIV+, 3.77% in HIV- vs 7.41% in PrEP) and Ct (7.41% in HIV+, 7.41% in PrEP vs 1.89 % in HIV-).

Discussion and Conclusions

Sexually transmitted pathogens were detected in 34 of the 56 (60%) evaluated patients. Data highlighted that Uu, Mh, Mg and Ct were the most etiological agents responsible for STIs in MSM selected population attending STDs clinic. Positivity for Uu was observed in 15 of 56 (26.78%) of the patients studied. Furthermore, HIV+ patients were affected by Mh infections.

Moreover, Ct infection was revealed equally in both HIV+ and HIV- in PrEP subject as reported in the results seen above. The study is unaffected by a limitation due to a small amount of samples collected in the time considered.

42 - Nontyphoidal Salmonella gastroenteritis in children: a preliminary study on antibiotic resistance.

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Nontyphoidal Salmonella gastroenteritis in children: a preliminary study on antibiotic resistance.

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Introduction

Nontyphoidal *Salmonella* (NTS) is one of the most common causes of acute bacterial gastroenteritis in children. Since multidrug-resistance NTS is an emerging health problem, the aim of this study was to evaluate the incidence and antimicrobial susceptibility of NTS isolated from diarrheic paediatric patients admitted to Giovanni XXIII Hospital, Bari, Italy.

Materials and methods

Stool samples were collected from 466 paediatric patients (range 0-23 years) from August 2021 to April 2022. *Salmonella enterica* was identified using MALDI-TOF mass spectrometry (Vitek® MS, BioMerieux), serotyped by slide micro-agglutination and tested for antimicrobial susceptibility to using Vitek® 2 system (BioMerieux).

Results

Thirty-nine (8%) stool samples were positive for *NTS* and *Salmonella* serovar Typhimurium was the most dominant serotype (75,67%). Other isolated serotypes were: *Salmonella* Oslo, *Salmonella* Derby, *Salmonella* Stradford, *Salmonella* Borgon, *Salmonella* Detroit, *Salmonella* Strathcona. The highest number of positive samples was found in patients with age between 0-6 years (70,2%), whereas 29,8% in patients older than 10 years. For *Salmonella* Thyphimurium, 26/28 (92,8%) isolates were resistant to ampicillin and amoxicillin-clavulanic, 4 (14.28%) to trimethoprim-sulfamethoxazole, 1 (3.57%) to ceftazidime, the current first line treatment. All isolates were susceptible to carbapenems and fluoroquinolones. For the other *Salmonella* serovars, 33% of isolates were resistant to ampicillin and amoxicillin-clavulanic and all susceptible to trimethoprim-sulfamethoxazole, ceftazidime, carbapenems and fluoroquinolones.

Discussion and Conclusions

Salmonella Thyphimurium is the most prevalent serotype responsible for NTS acute paediatric gastroenteritis and the most vulnerable patients are the children with age less than 6 years.

Although multidrug resistance has not been detected in NTS isolates, prudent use of antimicrobials is recommended because the treatment of NTS infections is not always required ensuring the long-term use of available antibiotics.

44 - Pilocarpine interferes with sphingolipids metabolism in Candida albicans.

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Pilocarpine interferes with sphingolipids metabolism in *Candida albicans.*

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Candida albicans is the most common human fungal pathogen with an estimated crude mortality rate of 40%. The ability of the organism to filament and to produce biofilms are important virulence factors, responsible for tissue invasion and antifungal tolerance, respectively. Pilocarpine hydrochloride (PHCl), a muscarinic receptor agonist, has been shown to inhibit *C. albicans* biofilm formation and *in vivo* pathogenicity in the *Galleria mellonella* model, but the mechanism underlying this effect is still unknown.

Due to the sphingolipids' (SLs) role in the yeast cell wall integrity signaling, filamentation and virulence, we investigated the possible effect of PHCl on *C. albicans* sphingolipid content.

Lipids from pellets of planktonic and biofilm-organized cells of *C. albicans* strain SC5314, treated or not with PHCl 25 M, were isolated by extraction with methanol/chloroform mixture coupled to alkaline methanolysis for phospholipids removal. The sphingolipids profile was obtained by liquid chromatography tandem mass spectrometry (LC-MS/MS) using electrospray ionization.

Ceramide content was found reduced in both untreated and PHCl-treated biofilm-organized cells, despite no differences in the dihydroceramide precursor. On the contrary, PHCl reduced Cer concentration in planktonic cells. Phytoceramide (PHC) production was increased by PHCl in both planktonic and sessile cells, whereas the alpha-hydroxylated PHC was enriched only in treated sessile cells. Inositol phosphoryl ceramide (IPC), the precursor of mannosilated IPCs (MIPC) reported to be involved in *Candida* morphogenesis, was significantly higher in biofilms and not affected by PHCl treatment.

Biofilm itself causes severe sphingolipid content remodeling. PHCl seems to exert a modulatory effect on SL pathway in both sessile and planktonic cells. Further studies encompassing other sphingolipids, such as MIPC and $M(IP)_2C$, are necessary to elucidate whether the PHCl-driven reduction in filamentation and biofilm formation could be SL-mediated.

45 - KPC-producing Klebsiella pneumoniae spread: genotypic and epidemiological characterization of clinical strains from Marche region

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KPC-producing *Klebsiella pneumoniae* spread: genotypic and epidemiological characterization of clinical strains from Marche region

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Introduction: The global spread of carbapenem resistant Gram-negative bacteria represents a worrying public health threat as carbapenems were one of the few therapeutic options for extended-spectrum beta-lactamase-producing strains. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are the most important one in severe nosocomial infections. We characterized *K.pneumoniae carbapenemase*-producing *K.pneumoniae* (Kp-KPC) from bloodstream infections from Marche region hospitalized patients, Italy, to evaluate the resistance rate to last resort antibiotics, the resistance mechanisms involved in antibiotic resistance beside carbapenems and highlight the presence of already widespread clones.

Materials and methods: A total of 83 Kp-KPC were collected from positive blood cultures from Ancona and Pesaro hospitals between January 2016 and December 2019. The presence of bla_{KPC} gene was confirmed by PCR experiments. MICs to colistin and ceftazidime-avibactam (CZA) were performed with the broth microdilution method. *GenElute bacterial genomic DNA* kit was used for total DNA extraction. All strains were typed by XbaI-PFGE: one strain for each pulsotype was subsequently selected and subjected to S1-PFGE and whole genome sequencing (WGS) by the Oxford Nanopore Technology. *Canu* and *Unicycler* were used for reads assembly while *BLAST* and *Center for Genomic Epidemiology* for data analysis.

Results: We found 24 out 83 isolates (28,9%) were colistin resistant (MIC > 2 mg/L). This resistance rate was decreasing in Ancona and Pesaro in the last year of study (2018: 36,4%; 2019: 11,8%). None CZA-resistant clone was detected. PFGE showed 29 pulsotypes with 3 major clusters: among them 2 were common in both hospitals, while the last one was spread only at Pesaro hospital and appeared in 2019. WGS highlighted 7 different sequence types (STs). The most represented were ST512 (71,1%) and ST307 (16,9%) shared by all the areas studied. ST307 showed a significant increase from 9,1% in 2018 to 58,8% in 2019 while ST512 passed from 68,2% to 29,4% respectively. KPC-3 was the most encoded allelic variant: only 4 KPC-2 isolates were detected, belonging to ST307.

Discussion and conclusions: The decreasing trend in colistin resistance in 2019 can be linked to both the administration of new therapeutic agents (CZA) and the success of different STs. Anyway, the shift from ST512 to ST307 should be worryingly since this clone is known for being more virulent and for having a greater number of resistance determinants. It is important to monitor the spread of ST307 to trace its epidemiological evolution and diffusion. Conscious use of CZA is also essential to prevent the appearance of resistant phenotypes.

47 - Re-sensitizing Methicillin-resistant Staphylococcus aureus to antimicrobials

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Re-sensitizing Methicillin-resistant *Staphylococcus aureus* to antimicrobials

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Introduction: Antimicrobial resistance (AMR) has become a global health concern in livestock and human settings. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common antibiotic-resistant bacteria, which has been classified as a high-priority pathogen for new antibiotic development. In the battle against AMR, re-sensitizing MRSA to existing antimicrobials might be a promising approach, as the full expression of the resistance phenotype requires helper enzymes that, together with the resistance genes, are essential for displaying resistance. In this study, we seek to identify those helper genes/proteins involved in oxacillin and tetracycline resistance in MRSA, which could serve as targets to restore susceptibility to these antimicrobials.

Materials and Methods: Large-scale transposon mutant library of MRSA ST398 was screened following separate exposure to ½ minimum inhibitory concentration of target drugs using transposon-directed insertion site sequencing technology (TraDis), which allows the identification of metabolic enzymes necessary to express the resistance to oxacillin and tetracycline. Using the Bio::TraDIS pipeline, we measured mutant depletion to compare the insertion density in treated and untreated cultures and assess statistically an eventual under-representation of transposon insertions in non-essential genes. In addition, the identified genes were re-annotated and functionally characterized using the Uniprot database.

Results: The assay revealed 51 and 141 non-essential genes that might contribute to oxacillin and tetracycline resistance, respectively. The statistical analysis of each gene (log2 Fold Change < -2, and p-value < 0.05) and its involvement in biological processes led to selecting a set of genes to be validated by gene knockout.

Conclusion: Transposon mutagenesis can provide an unprecedented insight into bacterial resistance and its secondary resistome**.** This work presents a preliminary list of secondary resistance genes to oxacillin and tetracycline in *Staphylococcus aureus*. These genes may be potential targets for developing new "helper" compounds that may restore the efficacy of the tested antimicrobials.

49 - Implementation of a selective Chromatic medium for active surveillance of ceftazidime-avibactam resistance during COVID-19 pandemic: outbreaks and diagnostic issues

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Implementation of a selective Chromatic medium for active surveillance of ceftazidime-avibactam resistance during COVID-19 pandemic: outbreaks and diagnostic issues

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Introduction: Acquired resistance towards ceftazidime-avibactam (CAZ-AVI) is increasingly reported. Several mechanisms can be involved, but mutations in the **Ω**-loop region of β-lactamases are the most described. Herein, we assessed the implementation of Chromatic Super CAZ/AVI® medium in rectal swab surveillance cultures in a geographic area with endemic distribution of KPC-producing *Klebsiella pneumoniae* (KPC-Kp).
Methods: Routine rectal swabs collected from Intensive Care Unit (ICU) and non-ICU patients were screened for carbapenemase-producing Enterobacterales (CPE), carbapenems-resistant Gram-negative organisms (CR-GN) and CAZ-AVI-resistant organisms by Chromatic CRE and Super CAZ/AVI® media.
Results: Among the 1839 patients screened, 146 (7.9%) were found to be colonized by one or more CPE and/or CR-GN isolates during hospitalization. Overall, among colonized patients the most common bacteria encountered were KPC-producing Enterobacterales (n= 60; 41.1%), meropenem-resistant *Pseudomonas aeruginosa* (n= 41; 28.1%) and meropenem-resistant *A. baumannii* (n= 34; 23.3%). Among patients colonized by KPC-producing Enterobacterales, thirty-five (58.3%) had CAZ-AVI-resistant strains. A 30.5% rate of faecal carriage of CAZ-AVI-resistant KPC-producing *K. pneumoniae*, substantially higher than that of susceptible isolates (2.8%), was observed in the COVID-19 ICU. Twenty-one out of 22 CZA-resistant KPC-Kp strains isolated by COVID-19 patients expressed an ESBL phenotype and showed susceptibility toward carbapenems. All phenotypic carbapenemase detection methods (CarbaNP, Disc Diffusion Synergy test [KPC, MBL and OXA-48 Confirm Kit, Rosco Diagnostica], modified carbapenem inactivaction method [mCIM], and lateral flow immunoassay [NG-Test CARBA 5- NG Biotech, France; RESIST-5 O.O.K.N.V- Coris Bioconcept, Belgium] failed to detect KPC carbapenemases conferring CZA resistance. Cluster analysis using Fourier-transform infrared spectroscopy on IR Biotyper system showed that 19 out of the 21 CZA-resistant KPC-Kp isolates belonged to a single outbreak clone.
Prevalence of faecal carriage of metallo-β-lactamases-producing organisms was low (0.5% and 0.2% for Enterobacterales and *P. aeruginosa*, respectively). Chromatic Super CAZ/AVI® medium showed 100% sensitivity in detecting CPE or CR-GN isolates resistant to CAZ-AVI regardless of both MIC values and carbapenemases content. Specificity was 86.8%.
Conclusion: The Chromatic Super CAZ/AVI® medium might be implemented in rectal swab surveillance cultures for identification of patients carrying CAZ-AVI-resistant organisms to contain the spread of these difficult-to-treat pathogens. Molecular testing are necessary to confirm expression of KPC variants associated with CAZ-AVI resistance.

51 - Cutaneous staphylococcal infection in immunocompromised site during nivolumab treatment for non-small cell lung cancer: a first case report

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Cutaneous staphylococcal infection in immunocompromised site during nivolumab treatment for non-small cell lung cancer: a first case report

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Introduction Immunotherapy in oncology is supplanting traditional therapies thanks to the specificity of action and the limitation of side effects. Despite its high efficacy, side effect as bacterial infection has been reported, especially pneumonia and urogenital tract infections. Bacterial skin and soft tissue infections (SSTI) represent one of the most important differential diagnoses in patients presenting with reddened and swollen skin and soft tissue. Among these infections, cellulitis (phlegmon) and abscesses are the most frequent. In most cases, they present as localized forms with possible contiguous spread, but in immunocompromised patients multifocal manifestation is frequent. We report a unique case report, of our knowledge, of a diffuse cutaneous infection with a phlegmon, in immunocompromised cutaneous site, in a patient treated with nivolumab for non-small cell lung cancer (NSCLC).

Materials and Methods A 64-year-old, smoker male patient became at our attention for cutaneous lesions at different evolution level in the left arm (three phlegmons), on the left side (erythematous plaque) and on the right wrist (scaly redness papule), all localized on a tattoo. To identify the etiopathologies agents, we performed clinical visit, complete blood test and cutaneous swabs.

Results Blood tests don't underline systemic infection or other concomitant comorbidities, while microbiological cultures and gram stains highlight a gram-positive infection caused by methicillin-susceptible but Erythromycin-resistant (ER-R) clindamycin-resistant CL-R) and gentamicin-resistant (GE-R) *Staphylococcus aureus*. Systemic treatment with clarithromycin 500 mg/day for 15 days and topical fusidic acid twice daily for 30 days was prescribed, with complete resolutions of all cutaneous lesions. No adverse events were reported.

Discussion and Conclusions Despite immunotherapy is being a milestone in oncologic treatment, more than the spectrum of immune-mediated toxicities from these agents need to be investigate. This report highlights the importance of considering lifestyle and cutaneous alteration before starting immunotherapy. Moreover, this case also emphasizes the potential value of dermatological screening in patients treated with Programmed death 1 (PD-1) checkpoint inhibitors and underlines how immunocompromised cutaneous site could be involved during immunotherapies.

54 - Introduction of probiotic-based sanitation in the emergency ward of a children's hospital during the COVID-19 pandemic

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Introduction of probiotic-based sanitation in the emergency ward of a children's hospital during the COVID-19 pandemic

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Introduction: The massive use of disinfectants to prevent COVID-19 transmission might worsen the antimicrobial resistance (AMR) threat, especially in the hospital environment, possibly leading to future AMR pandemics. However, the control of microbial and viral contamination is crucial in hospitals, since hospital microbiomes can cause the healthcare-associated infections (HAIs), which are particularly frequent and severe in pediatric wards, due to children high susceptibility.
We have previously reported that a probiotic cleaning hygiene system (PCHS) can stably decrease bacterial pathogens and their AMR in the hospital environment, reducing associated HAIs in adult hospitals. Since we recently showed that PCHS can also inactivate enveloped viruses in vitro, here we aimed to test the effect of PCHS in the emergency room (ER) of a children's hospital during the COVID-19 pandemic.
Material and Methods: PCHS was used for routine ER sanitation for 2 months in substitution of the conventional chemical disinfection. The environmental bioburden was characterized before and during PCHS application (at 2, 4, and 9 weeks after PCHS introduction). Microbial contamination was monitored by both conventional culture-based CFU count and molecular assays, including 16S rRNA NGS for bacteriome characterization and microarrays for the assessment of the resistome of the contaminating population. The presence of SARS-CoV-2 was also monitored by PCR.
Results: PCHS usage was associated with a stable 80% decrease in bacterial/fungal pathogens compared to the levels detected with chemical disinfection ($P < 0.01$), accompanied by an up to 99% decrease of AMR genes ($Pc < 0.01$). Besides, SARS-CoV-2 was stably absent in PCHS-treated environments. The PCHS effects were reversed when reintroducing chemical disinfection, which counteracted the action of the PCHS.
Discussion and Conclusions: Since the control of microbial contamination continues to be a major issue in the management of the pandemic emergency, collected data suggest that PCHS may be successfully used to control virus spread without simultaneous worsening of the AMR concern.

55 - Genomic fingerprinting of a Mycobacterium bovis BCG causing a severe vertebral osteomyelitis following BCG instillation in a bladder cancer patient

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Genomic fingerprinting of a *Mycobacterium bovis* BCG causing a severe vertebral osteomyelitis following BCG instillation in a bladder cancer patient

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Introduction Bacillus Calmette-Guérin (BCG), an attenuated derivative of the virulent strain of *Mycobacterium tuberculosis* var. *bovis* (*M. bovis*), is globally used for vaccination against tuberculosis or treatment of non-muscle invasive bladder cancer, since it triggers an innate immunological reaction that is protective against tumor recurrence and progression. Nevertheless, adverse reactions (ARs) to BCG immunotherapy can be observed after months or years, especially in immunocompromised patients, including lymphadenitis, osteomyelitis, and disseminated BCG infections. Nosocomially acquired, catheter-related BCG infections have been also documented. At present, genetically diverse BCG sub-strains exist, and their genetic diversity may influence the immunization efficacy and severity of disseminated infections. The limited number of cases, however, prevents a fine assessment of possible genetic variations occurring in BCG strains associated with ARs. Here we report on the genomic characterization of a clinical BCG vaccine strain causing a severe vertebral osteomyelitis following BCG immunotherapy.

Materials and Methods *M. bovis* BCG-GE was isolated (BACTED MGIT, BD, US) in September 2021 from a biopsy sample of a bladder cancer patient admitted to a tertiary-care hospital in Albenga (Savona, Italy), previously subjected to BCG instillation with the OncoTice strain (Merck, US). Initial identification was carried out by MALDI-TOF mass spectrometry (Becton Dikinson, US). Whole-genome sequencing (WGS) was performed using the Oxford Nanopore MinION system (ONT, UK) and raw reads were assembled using Flye. *In silico* identification was performed using the Pathogenwatch webserver, while global genomic comparative analyses were carried out using snippy, iq-tree, and Mauve.

Results Long-reads sequencing resulted in a complete circular genome of BCG-GE (total length: 4,317,915 bp; coverage: 40x; GC%: 65,7). A WGS-based identification as *Mycobacterium tuberculosis* var. *bovis* BCG was achieved. Global phylogenetic analysis, including 143 *M. bovis* genomes, revealed a close clonal relationship with the BCG Tice vaccine strain. A pairwise comparison of BCG-GE and BCG Tice genomes revealed several genetic alterations in gene products likely involved in bacterial growth, host adaptation and virulence processes (e.g. ESX-type secretion systems, MmpL- and MCE-type transport systems, PE/PPE and MCE proteins).

Discussion and Conclusions To the best of our knowledge, we report the first WGS-based comparative analysis of the BCG-Tice vaccine strain and a BCG Tice clinical isolate causing a severe vertebral osteomyelitis. Present results provide a background genomic information that can be exploited to investigate the genetic heterogeneity of BCG strains associated with ARs.

64 - Antimicrobial activity of WMR-K against Neisseria meningitidis

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Antimicrobial activity of WMR-K against *Neisseria meningitidis*

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Introduction. Antimicrobial peptides (AMPs) represent an attractive alternative to conventional drugs for the development of new antimicrobials since they have shown to have broad-spectrum activity towards bacteria, fungi and viruses, furthermore, the resistance mechanisms towards them are limited. Although AMPs being highly diverse in sequence, length and structure, they present similar mechanisms of action, which involve the disruption of bacterial membrane integrity. The antimicrobial WMR peptide, which is a modification of the native sequence of myxinidin, a marine peptide isolated from the epidermal mucus of hagfish (*Myxine glutinosa* L.), showed a potent antimicrobial activity against a wide range of bacteria and yeast. A later modification of the WMR sequence, the analogue WMR-K, was developed to enable peptide covalent binding to self-assembled peptide fibers for intracellular delivery. The aim of this study was to investigate the antimicrobial activity of the WMR-K peptide against *Neisseria meningitidis* strains. **Materials and Methods.** In order to identify the antimicrobial activity of the WMR-K peptide against meningococccus, it was evaluated the minimum concentration of molecule capable of inhibiting the growth of the microorganism (MIC) and the minimum concentration of molecule capable of killing the microorganism (MBC) against selected *N. meningitidis* strains (serogroups A and C) testing increasing concentration of analogue (from 1µM to 100µM). Time kill kinetics of WMR-K were also evaluated against selected *N. meningitidis* strains: in detail, values corresponding to MBC and 2, 4, 8 -fold the MBC were tested. **Results**. The obtained experimental data highlighted the efficacy of WMR-K analogue against meningococcal strains with MIC and MBC values ranging from 10µM to 90 µM. Moreover, time kill kinetics assays have defined that the analogue exerts its activity within the first hour of contact. **Discussion and Conclusion.** Although they

are preliminary data, the results of the antimicrobial activities suggest a promising capacity of low concentrations of WMR-K to inhibit *N. meningitidis* strains belonging to widespread serogroups. In the next future, the synergistic effect of the analogue with standard antibiotic therapies will be also evaluated.

65 - Effect of pomegranate peel extracts against ESKAPE

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Effect of pomegranate peel extracts against ESKAPE

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Introduction: The improper use and abuse of antibiotics has led to the increase and spread of new mechanisms of multi-drug resistance with a consequent decrease of the effectiveness of standard antibiotic therapies. The multi-drug resistant (MDR) strains entail a greater difficulty of treatment with a strong possibility of therapeutic failure. This phenomenon currently represents a real emergency in the health field which requires alternative routes to fight infections such as the identification of new molecules with antimicrobial activity capable of acting on MDR strains. The aim of the present study was to evaluate the antimicrobial activity of hydroalcoholic pomegranate peel extract thanks to its characterized content of polyphenolic compounds and antioxidant activity strength. **Materials and methods:** The agar diffusion method was conducted to evaluate the inhibitory spectrum of extracts against ESKAPE group: *Staphylococcus aureus* ATCC, *Pseudomonas aeruginosa* ATCC and MDR clinical strains such as Methicillin resistant *S. aureus* (MRSA), *P. aeruginosa* MDR, *Acinetobacter baumannii* MDR and *Klebsiella pneumoniae* carbapenemase producer (KPC). The susceptibility of selected isolates to different concentrations of hydroalcoholic pomegranate peel extracts was determined using microdilution assay. **Results:** The pomegranate peel extracts effectively inhibit the growth and survival of all bacterial strain tested, as demonstrated by the diameters of inhibition zones exerted by low amount of extract (>10mm).

Accordingly, quantitative evaluation of the antimicrobial activity of pomegranate peel extract against selected ESKAPE strains showed high inhibitory activity against *S. aureus* ATCC and MRSA with a minimum bactericidal concentration (MBC) of 16 μg/μl; while against *P. aeruginosa ATCC* and relative MDR clinical isolates, the MBC values obtained were ranging from 40 to 80 μg/μl. Instead for *A. baumannii* MDR and *K. pneumoniae* KPC the MBC values obtained are respectively 20 μg/μl and 200 μg/μl. **Discussion and Conclusion:** The preliminary data obtained by *in vitro* microbiological assay, demonstrate that the hydroalcoholic pomegranate peel extract is able to inhibit the bacterial viability of reference and MDR strains belonging to the ESKAPE group. The promising experimental obtained data suggest to evaluate the synergistic activity with standard antibiotic therapies that could restore the antimicrobial drug efficacy.

66 - Quality assessment of surface and groundwaters in Lombardy, Italy: detection and characterization of ESBLs-producing Enterobacterales

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Quality assessment of surface and groundwaters in Lombardy, Italy: detection and characterization of ESBLs-producing *Enterobacterales*

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Introduction: This study aimed to screen the presence of Extended-Spectrum beta-Lactamases (ESBLs) and/or carbapenemases-producing *Enterobacterales* in surface/groundwaters in Lombardy, Italy. **Materials and Methods:** On March 2021, n=31 water samples were collected from n=14 natural and n=9 artificial springs, n=6 wastewater treatment plants (WWTPs), and n=2 sites upstream and downstream the Ticino River, within the Vigevano (PV) and Abbiategrasso (MI) areas. 50ml of water were filtered through 0.45µm pore size membranes. The filters were placed on MacConkey Agar (MCA), and selective MCA (cefotaxime 0.5mg/L-2mg/L; meropenem 0.25mg/L-4mg/L). Species identification and antibiotypes were obtained by MicroScan autoSCAN-4 System (EUCAST 2020 breakpoints). BL, *aac(6')-Ib-cr* and *qnrB/S* genes were detected by PCR and sequencing. The concentration in the sampling sites of cephalosporines, fluoroquinolones, sulfonamides, macrolides, and tetracycline were also investigated. **Results:** A total of 264 Gram-negative isolates have been identified. The bacterial genera *Acinetobacter* spp. (n=56), *Pseudomonas* spp. (n=34), *Escherichia* (n=28) and *Aeromonas* spp. (n=24) were mostly found. Non-wild type (NWT) species belonging to *Enterobacterales* were further characterized. The 31.1% (n=9) of the NWT *Escherichia coli* isolates were ESBL-producers (6/9 *bla*CTX-M-15, 2/9 *bla*CTX-M-1, and 1/9 *bla*SHV-type positive). Resistance to trimethoprim-sulfamethoxazole occurred in NWT *E. coli* with the same percentage (31.1%); in 14.3% (n=4) of the strains, fluoroquinolones resistance *qnrS* gene related. An aminoglycoside-resistant isolate showed *aac(6')-Ib-cr* gene positivity. Six *E. coli* (5/6 ESβL and 1/6 non-ESβL-producer) were MDR. Among the 16 NWT *Klebsiella* spp. isolates: n=10 were *K. pneumoniae*; n=5 *K. oxytoca* and n=1 *K. ozaenae*. 4/16 of the above isolates (n=3 *K. pneumoniae* and 1 *K. oxytoca)* resulted MDR. These four strains harbored also the *aac(6')-Ib-cr* gene. A total of n=22 NWT strains of *Citrobacter* spp. were identified: 50% *C. freundii*, 4/22 *C. youngae* and *C. braakiii*, and 3/22 *C. farmerii*. 31.8% of *Citrobacter* spp. showed acquired resistance to fluoriquinolones (in *C. freundii aac(6')-Ib-cr* gene mediated). Interestingly, trimethoprim was ubiquitously found, while all the other antibiotics showed a dectable presence in the majority of the sampling sites. A high microbial load was found in particular in those sampling sites close to WWTPs. **Discussion and Conclusions:** A low levels antibiotic contamination observed at most sites, together with the *bla*CTX-M-type, *aac(6')-Ib-cr*, and *qnrS* detection in MDR *Enterobacterales* from water compartment in Lombardy highlights the need of undertaking proper remediation measures, as planned in the CE4WE "Hub Research and innovation" project.

69 - The protein HslJ boosts Acinetobacter baumannii survival against oxidative stress

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The protein HslJ boosts *Acinetobacter baumannii* survival against oxidative stress

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1. Introduction: *Acinetobacter baumannii* is a life-threatening opportunistic pathogen that causes different infections, including ventilator-associated pneumonia. Due to its ability to sense and respond to the environmental stresses, *A. baumannii* can survive in hospital settings and persist during host infections. In a previous study, we identified the periplasmic protein encoded by the ABUW_2868 locus (*hslJ* gene) as being upregulated during imipenem exposure and hypothesized that it participates in the protective response against the oxidative stress. 2. Materials and Methods: A *hslJ* mutant was attained from strain AB5075. Hydrogen peroxide assays and macrophage infections were used to determine the survival rates by colony forming units (CFU/ml) of the *hslJ* mutant in comparison with the wild type AB5075 strain. Surface hydrophobicity, biofilm-forming activity and motility in the *hslJ* mutant were also analyzed. *hslJ* transcriptional regulation is under evaluation using trans-activation experiments and electrophoretic motility shift assays (EMSA). 3. Results: In comparison with the wild type strain, the absence of *hslJ* gene caused a significant drop in cell survival rates of the mutant strain both upon exposure to exogenous H_2O_2 and macrophage infection. Moreover, compared to the wild type strain, the *hslJ* mutant displayed a decrease in biofilm formation and motility abilities although had a higher surface hydrophobicity. Furthermore, preliminary results suggest that expression of *hslJ* is under the control of the master regulator in defense against oxidative stress, OxyR. 4. Discussion and Conclusions: The *hslJ* gene could be part of the regulon of OxyR. Being the HslJ protein located into the periplasm, it represents the first line of defense that confers resistance to oxidative stress that *A. baumannii* faces both in disinfectants used in the hospital environments and innate immune cells during infection of the lungs. These findings provide a basis to develop novel therapeutics against this new target.

72 - Antimicrobial resistance during COVID-19 pandemic: microbial surveillance at "Cristo Re" Hospital during 2020-2021

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Antimicrobial resistance during COVID-19 pandemic: microbial surveillance at "Cristo Re" Hospital during 2020-2021

Introduction

With the advent of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, an increase in the incidence of antimicrobial resistance (AMR) related to broad-spectrum antibiotics overuse in patients with coronavirus disease-19 (COVID-19) has been documented. On the other hand, new preventive measures against COVID-19 diffusion could have affected microbial infections in healthcare settings. Our aim was to assess AMR incidence among the patients attending "Cristo Re" Hospital, Rome, for routine visit and prolonged hospitalization from January 2020 to December 2021.

Material and Methods

Biological samples were analyzed for common microorganisms by standard methods and procedures for the traditional microbiological investigation. The microorganisms growing on plates were identified and tested for antimicrobial susceptibility by Vitek2 automated system (Biomerieux, France). AMR detection was eased by the use of chromogenic plates for surveillance rectal and pharyngeal swabs (Biomerieux, France). AMR was always confirmed by disk diffusion method (Kirby-Bauer). Statistical analyses were performed with Statistical Package for Social Science (SPSS) version 25.

Results

We registered a total of 316 pathogenic isolates in 2020 and 366 in 2021. A lower number of multidrug resistant (MDR) isolates was registered during 2021 (n=88, 24.0%) compared to 2020 (n=122, 38.6%) ($p<0.001$). Analyzing bacterial isolates individually, a higher incidence emerged for methicillin resistant *Staphylococcus aureus* (MRSA) in 2020 against 2021 (60.0% vs 20.0%, $p=0.002$), while no significant difference emerged for vancomycin resistant *Enterococcus* (VRE) (18.0% vs 9.3%), extended spectrum beta lactamase *Escherichia coli* and *Klebsiella pneumoniae* (ESBL) (36.1% vs 24.2%; 20.7% vs 13.3%), carbapenem resistant *K.pneumoniae* (36.1% vs 20.4%), MDR *Acinetobacter baumanni* cplx (91.3% vs 90.9%), MDR *Pseudomonas aeruginosa* (41.2 vs 33.3) and SXT resistant *Stenotrohomonas maltophilia* (11.1% vs 16.7%) ($p>0.05$).

Conclusion

The retrospective analysis of the microbiological data collected during the first two years of the pandemic suggests a reduction of AMR isolates comparing 2020 to 2021. The adoption of preventive measures against COVID-19 transmission, as the use of adequate personal protective equipment, increased attention to hand sanitation, frequent change of gloves and gowns, segregation of patients with respiratory symptoms and universal masking policies could have had an impact on AMR incidence. In addition, these preliminary results could be partially explained by the recovery of the control practises against MDR isolates diffusion inside hospital units after the emergency of the first year of COVID-19 pandemic. Further investigations are necessary to confirm this trend in our hospital structure.

74 - Antibacterial and antibiofilm activity in artificial sputum medium of Lactobacilli against Pseudomonas aeruginosa strains isolated from CF patients

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Antibacterial and antibiofilm activity in artificial sputum medium of Lactobacilli against *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients

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Introduction. The therapy of lung infections sustained by *P. aeruginosa* in cystic fibrosis (CF) patients is still problematic due to the presence of a sticky mucus in airways and to the formation of biofilm, which exhibits increased antibiotic tolerance. The use of probiotics has demonstrated efficacy in treating human intestinal disorders, and it is being proposed in a growing number of other than digestive clinical applications including respiratory tract infections. The presence of lactobacilli in the airways of CF patients has been recently demonstrated. In this study we evaluated the ability of probiotic strains to grow in artificial sputum medium (ASM), mimicking the CF lung conditions, and to affect the planktonic and biofilm growth of *P. aeruginosa* in the same conditions.

Materials and methods. Probiotic strains of *Lactobacillus* genus isolated from commercial products and three *P. aeruginosa* strains (CF1: not mucoid; CF4 and CF11: mucoid) isolated from the sputum of CF patients were used in this study. Ability of *Lactobacillus* probiotic strains to grow in ASM and to affect planktonic growth of CF1 and CF4 strains was evaluated by CFU count after 24h of incubation/co-incubation in such medium. The ability of lactobacilli to aggregate with *P. aeruginosa* was tested incubating equal volumes of lactobacilli and CF1 or CF4 strains. Aliquots of coaggregating mixtures were withdrawn at regular intervals to calculate the aggregation index by spectrophotometric measurements at a wavelength of 600nm. The antibiofilm effect of lactobacilli on CF1, CF4 and CF11in ASM was evaluated by crystal violet staining or by CFU count.

Results. Among six lactobacilli strains tested, *L. plantarum* (Lp) and *L. rhamnosus* (Microbiosis) (Lr) demonstrated the highest ability to grow in ASM. Lp was also able to partially inhibit the growth of CF1 and CF4 strains in ASM and to form aggregates with both *P. aeruginosa* strains. Both Lp and Lr were able to reduce the biomass of mature biofilms of the two *P. aeruginosa* strains tested, whereas they did not inhibit the formation of biofilms by the same strains. Interestingly, when mature biofilms of CF1 and CF11 strains were sequentially exposed to Lp and tobramycin a significant decrease in the number of CFU was observed compared to the biofilms treated with tobramycin only.

Discussion and Conclusions. The ability of Lp to reduce the viable count of *P. aeruginosa* in ASM, either in planktonic or biofilm form of growth, to strongly aggregate with it, and to synergize with tobramycin suggest such probiotic strain as a promising candidate as adjuvant treatment of pulmonary infections in CF patients. *The study received support from the Italian Cystic fibrosis research foundation, Project FFC#13/2021.*

77 - The One-year Comparative study between molecular and cultural cutaneous swab tests during surveillance program of Candida auris in ICUs of San Martino Hospital (July 2021-July 2022)

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The One-year Comparative study between molecular and cultural cutaneous swab tests during surveillance program of *Candida auris* in ICUs (Intensive Care Units) of San Martino Hospital (July 2021-July 2022)

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INTRODUCTION *C. auris* is a multi-drug resistant nosocomial fungus emerged as a 'serious threat' worldwide, associated with nosocomial outbreaks and high rate of mortality in critically patients. Since 2019, the San Martino Hospital (HSM, Genoa, Italy) experienced a large *C. auris* outbreak likely fueled by the COVID-19 pandemic. In this , a rapid laboratory response is required for infection prevention and control strategies. We report the one-year data of a preliminary comparative study between cultural and molecular testing for *C. auris* skin colonization, in the context of a surveillance program adopted by HSM to prevent transmission of *C. auris*.
MATERIALS AND METHODS A total of 1124 paired (cultural and molecular) cutaneous swabs were collected from 619 patients admitted to HSM ICUs during the period July 2021–July 2022. Fungal identification was carried out by MALDI-TOF MS (Vitek MS; bioMérieux, France). For cultural analyses (CS), dry cotton swabs (Cliniswab DS, Aptaca Spa) were streaked onto Chromatic Candida agar plates (Liofilchem, Italy), while wet flocked swabs (eSwab®, Copan s.p.a, Italy) were used for molecular analyses (MS). Total DNA was extracted using the MagCore Plus II automatic extractor (RBC Bioscience Corp., Taiwan), and Real-Time PCR testing was performed using the RealCycler *C. auris* PCR Kit (Progenie Molecular SLU, Spain).
RESULTS Overall, 74/1124 (6.6%) swabs from 70 patients tested positive to both CS and MS (CS+/MS+); similarly, all negative CS swabs were also negative to MS (CS-/MS-). Discordant results were observed in 17 cases, testing CS-/MS+. Among these, a second swab pair was available in 6 cases and tested CS+/MS+ after 7-14 days (mean: 10.5±3.5) from the initial sampling, while positive *C. auris* cultures were observed in specimens of other 6 CS-/MS+ cases. In addition, 40/70 (57.1%) patients testing CS+/MS+ had a positive *C. auris* culture from other specimens within a month (mean: 13±9) from the initial sampling (invasive infections in 13/40 cases). A positive and a negative predicting value (PPV, NPV) of 81.3 and 98.4%, respectively, was calculated for the molecular approach.
DISCUSSION AND CONCLUSIONS Present results proved that molecular testing allows for a marked reduction of the turn-around time, lowered to 6-18h vs. 24-72h required from the conventional cultural approach to detect *C. auris* for surveillance purposes. PPV and NPV values were not entirely superimposable, however, consistent results (CS+/MS+ and CS-/MS-) were observed in most cases (98.5%). Further studies are needed to better evaluate the performances of molecular over cultural approaches. The rapid tracking of *C. auris* is of utmost importance to deliver effective responses aimed at containing its spread within the healthcare system.

78 - Development, characterization and antibacterial activity of polyether-co-amide matrix films incorporating choline-calixarene nanoassembly

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Development, characterization and antibacterial activity of polyether-co-amide matrix films incorporating choline-calixarene nanoassembly

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Introduction Calix[n]arenes are a family of phenolic-based macrocycles of great interest for their synthetic versatility and ability to self-assembly in nanostructured systems. In the search for novel antibacterial agents, we previously demonstrated that the micellar calix[4]arene amphiphile (Chol-Calix), bearing hydrophilic choline moieties and long hydrophobic aliphatic chains, is a nanocarrier for antibiotics (ofloxacin, tetracycline and chloramphenicol) and possesses intrinsic antibacterial properties also against antibiotic-resistant bacteria. To extend the research, here we develop a novel material by incorporating the Chol-Calix into a polyether-co-amide matrix (Pebax®2533) and study its physico-chemical and antibacterial properties.

Materials and Methods Flexible films based on Pebax®2533 loaded with Chol-Calix were prepared by solution casting method. The films were characterized for morphology, phase miscibility, thermal stability, gas transport, spectral properties, and Chol-Calix release. The antibacterial activity of the films, neat Pebax® and Pebax® blends loaded with Chol-Calix (0.5, 1, 5 wt%), was evaluated against *Escherichia coli* ATCC 10536 and *Staphylococcus aureus* ATCC 6538 at different time intervals (2, 4, 6, 8, 10, 24 h) by cell number evaluation and time kill plots construction. The effect on biofilm formation was estimated by biomass measurements. MTT assay was employed to investigate the interference on vitality of mouse embryonic fibroblast cells (NIH-3T3).

Results The thermal stability of the copolymer was not affected by the Chol-Calix incorporation, nevertheless it was detected an increase of crystallinity, gas permeability and wettability of the blend films according to the additive concentration. Leaching of Chol-Calix was tracked by release tests. Neat Pebax® and Pebax®-0.5 wt% Chol-Calix showed no significant antibacterial activity against both *E. coli* and *S. aureus*. Pebax®-1 wt% Chol-Calix displayed good antibacterial activity against *S. aureus*, with a reduction of 1.8 and 2.1 log CFU/mL observed at 10 and 24 h, respectively. A clear effect was observed with Pebax®-5 wt % Chol-Calix that reduced the number of viable *E. coli* cells of 2.57 and 2.66 log CFU/mL at 10 and 24 h, respectively. A similar trend was observed for *S. aureus* with a reduction of 2 log CFU/mL at 10 h that increased to 2.49 log CFU/mL at 24 h. Pebax®-5 wt % Chol-Calix showed biofilm biomass reduced (~30%) as compared to the neat polymer. The films were non-cytotoxic as revealed by MTT assay.

Discussion and Conclusions The results indicate the Pebax®/Chol-Calix combination as a promising approach for the development of novel biocompatible flexible antibacterial thin-films upgradable by loading antibacterial drugs in the Chol-Calix nanocarrier.

79 - Antimicrobial effectiveness of ozonated olive and sunflower seeds oils

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Antimicrobial effectiveness of ozonated olive and sunflower seeds oils

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Introduction

Drug-resistance represents one of the great plagues of our time, largely limiting the treatment of common infections and accounting for the urge in the development of new antibiotics or other alternatives. Noteworthy, the indiscriminate use of antibiotics is mostly responsible for the emergence of mutations that confer drug-resistance to microbes. Recently, beyond synthetic and green molecules studied to tackle microbial infections, ozone is raising interest for its unique biological properties in particular when dissolved in natural oils, which stabilize it. In fact, ozonated oils have been reported to act in a non-specific way on microorganisms hindering the acquisition of advantageous mutations resulting in resistance. Here, we focused on comparing the antimicrobial effect of two different types of ozonated oils over a panel of opportunistic and pathogenic microbes.

Materials and methods

Ozonated olive (OOO) and sunflower seeds (OSO) oils were preliminarily characterized by chemico-physical investigation and used in *in vitro* experiments. A panel of microorganism that usually cause common infections (i.e., *Candida albicans*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*) have been challenged with different ozonated oils concentrations to evaluate the antimicrobial resistance/susceptibility by agar diffusion and broth dilution tests. Cytotoxicity was also evaluated in keratinocytes and endothelial cells.

Results

In our experimental conditions, OOO and OSO showed a potent microbicidal effect especially against *C. albicans* and *E. faecalis*, with the intermediate concentrations showing the highest efficacy unexpectedly. However, further investigations are required to clarify the effect on the other microbes. Both OOO and OSO do not exert any cytotoxic effect at the active concentrations in both cell lines.

Discussion and conclusion

Our results indicate that these ozonated oils are not toxic for mammalian cells and exert potent antimicrobial effects on specific microorganisms.Therefore, they may be considered to integrate standard therapies in the treatment of common infections, likely overcoming drug resistance issues.

82 - Epidemiology of bloodstream infections and mortality rates in patients admitted to the Intensive Care Unit in the pandemic era

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Epidemiology of bloodstream infections and mortality rates in patients admitted to the Intensive Care Unit in the pandemic era

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Introduction: Bloodstream infections (BSIs) are defined by the presence of bacterial or fungal microorganisms in the bloodstream, which is demonstrated by positive blood cultures. Recent studies show that 20% of BSIs occur in the Intensive Care Unit (ICU) where SARS-CoV-2 infection has exacerbated this problem, regarding prognosis and mortality. The aim of this study was to assess the burden of BSI in ICU patients in terms of prevalence and mortality rates in our Teaching Hospital during the pandemic era.
Materials and Methods: A total of 82 SARS-CoV-2 positive and 229 SARS-CoV-2 negative patients with at least one positive blood culture, admitted to the ICU, were enrolled, in a period between 1st January 2020 and 31st December 2021. The diagnosis of COVID-19 was confirmed by rRT-PCR. Blood culturing was performed for 7 days using the BacT/ALERT VIRTUO system (bioMérieux) according to routine laboratory practice. Identification was carried out using MALDI-TOF (bioMérieux) and Vitek®2 System (bioMérieux). Antibiotic susceptibility testing was performed by conventional methods. Statistical analysis was conducted using the chi-square test (p-value ≤ 0.05).
Results: Out of 311 patients enrolled, 75% (235/311) were male, while 25% (76/311) were female, with a median age of 69 years (age range 16-93). SARS-CoV-2 positive patients showed a higher prevalence of BSIs with *A. baumannii cplx* (16% vs. 8%; p= 0.03), *Enterococcus spp*. (12% vs. 7%), *K. pneumoniae* (8% vs. 6%), *E. coli* (2% vs. 1%) and *Streptococcus spp* (2% vs. 1%). SARS-CoV-2 negative patients showed a higher prevalence of BSIs with coagulase-negative staphylococci (CoNS) (52%) versus 44% among SARS-CoV-2 positive patients, *S. aureus* (4% vs. 0%, p = 0.01) and *Pseudomonas spp.* (3% vs. 2%). In SARS-CoV-2 positive and negative patients *Candida spp.* isolated strains were similar. We estimated the mortality rate in ICUs for patients with BSIs to be 38%. Regarding the causes of death, pulmonary complications were associated with SARS-CoV-2 positive patients (98% vs. 52%; p = 0.04), while cardiac and other complications were associated with SARS-CoV-2 negative patients (28% vs. 2%; p = 0.0006 and 20% vs. 0%; p = 0.002, respectively). 18 patients with BSIs were subjected to Extracorporeal Membrane Oxygenation (ECMO).
Discussion and Conclusions: Our study shows a mortality rate in accordance with the data in the literature (between 20% and 40%). Furthermore, the most frequently isolated microorganisms were CoNS, associated with SARS-CoV-2 negative patients. However, the burden of deaths from pulmonary complications is associated with SARS-CoV-2 positive patients.

83 - Humoral Response to Microbial Biomarkers in Parkinson’s disease Patients

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Title: Humoral Response to Microbial Biomarkers in Parkinson’s disease Patients

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Introduction:

Parkinson’s disease (PD) is a neurodegenerative disorder with the accumulation of alpha-synuclein (α-syn)/Lewy bodies in the brain and -enteric nervous system. The etiology of the disease is not well understood but bacterial and viral infections may contribute to the etiopathogenesis of PD. It has been suggested that Gastrointestinal (GI) complications observed in PD patients may rise from bacterial dysbiosis and curly/αsyn deposits in the enteric nervous system. Enteric bacteria such as *E. coli*, and *Salmonella* secret curli, a functional amyloid peptide that is involved in adhesion to surfaces, cell invasion, and biofilm formation. However, these bacterial amyloids can initiate additional α-syn deposits through a different mechanism. In this study, we investigated the humoral response against α- syn, curli peptides, and various bacterial and viral immunogen peptides in PD patients and compared them with healthy controls (HCs) sera.

Materials and Methods: Polyclonal IgG antibodies (Abs) specific were detected against peptides derived from alpha-synuclein (α- syn $_{100\text{-}114}$), curli (csgA$_{133\text{-}141}$), *Porphyromonas gingivalis*; Pg (RgpA $_{800\text{-}812}$, Kpg$_{328\text{-}339}$), *Aggregatibacter actinomycetemcomitans* (LtxA1$_{429\text{-}445}$, LtxA2$_{64\text{-}80}$), *Mycobacterium avium* subsp. *paratuberculosis* (MAP 3865c $_{125\text{-}133,}$ MAP 1,4-a-gbp$_{157\text{-}173}$ and MAP_4027 $_{18\text{-}32}$), Epstein-Barr virus (EBNA1$_{400–413}$, BOLF$_{1305–320}$), and Herpes Simplex virus 1 (UI42$_{22\text{-}36}$) investigated by indirect ELISA in 51 serum samples from PD and 58 sex and age-matched HCs.

Results: Significant differences in antibody titers and positivity were observed for Kpg (82.3% vs. 10.3%) followed by RgpA (60.7% vs. 24.1%), CsgA (51% vs. 22.4%) and UI42 (43.1% vs. 25.8%) in PD compared HCs sera ($p< 0.001$). No significant difference was found in the antibody’s titers to other tested peptides in patients with PD compared to HCs. A *significant positive correlation* between OD values obtained by ELISA was observed for UI42 and CsgA ($r = 0.811$, $p < 0.0001$), Kpg and RgpA ($r = 0.659$, $p < 0.0001$) followed by LtxA1 and LtxA2 ($r = 0.653$, $p < 0.0001$). The correlation analysis between the HY scale (Hoehn and Yahr Scale) and LtxA1 ($r = 0.306$, $p < 0.028$) and HY and Kpg ($r = 0.290$, $p < 0.038$) was significantly positive.

Discussion and Conclusion: This study reports a significantly increased humoral response against Curli, Pg, and HSV1 in PD patients implying that they could be an important factor in the pathogenesis of the disease. In addition, a high positive correlation between UI42 and CsgA may suggest the hypothesis of the involvement of HSV1 in GI dysbiosis and biofilm formation. Therefore, the role of each individual pathogen and curli in PD needs to be further investigated.

Keywords: Parkinson’s disease; humoral response; Curli; *P. gingivalis*; *A. actinomycetemcomitans*; *Epstein–Barr virus*; M. *avium subspecies paratuberculosis*; *Herpes simplex virus*.

84 - Active surveillance for MDR bacteria and correlation with HCAI in patients attending to a University Hospital: a 5-year retrospective study

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Active surveillance for MDR bacteria and correlation with HCAI in patients attending to a University Hospital: a 5-year retrospective study

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Introduction: Healthcare associated infections (HCAI) are infections occurring in a patient undergoing medical treatment in a hospital or other healthcare facility which were absent at the time of admission. They are a serious burden on public health in terms of costs and patient management. MDR bacteria are main causes of HCAI and are associated with high mortality in critical wards. We aim to assess the prevalence of MDR bacteria isolated from nasal, pharyngeal and rectal surveillance swabs and their correlation with HCAI in the University Hospital, Catanzaro, Southern Italy.
Materials and Methods: In this retrospective study, from 1st January 2017 to 31st December 2021, a total of 2621 patients attending our Teaching Hospital were enrolled. MDR bacteria were isolated by conventional culture media from rectal and pharyngeal swabs, while MRSA was isolated from nasal swabs. Identification and antibiotic resistance testing were performed by Vitek®2 and Sensititre systems. Positive patients were stratified into two different groups: positive patients on hospital admission (group 1) and positive patients after 48 hours of hospitalisation (group 2). Statistical analysis was conducted using chi-square test (p-value ≤ 0.05).
Results: Over the course of five years, a total of 15.122 screening swabs were performed, with an increasing trend over the years (from 2648 in 2017 to 4630 in 2021). Among 2621 patients, 83% (2177/2621) were negative to MDR bacteria, 12% (240/2621) belonged to group 1, while 8% (204/2621) belonged to group 2. In the first three years, we observed a linear increase of patients who were positive both on admission to our hospital and with HCAI (from 7% and 5% in 2017 to 13% and 9% in 2019, respectively). In the last two years the trend is strongly decreasing: 11% and 10% in 2020, 7.2% and 6.5% in 2021, respectively. MDR strains isolated from rectal (7.7%) and pharyngeal (3.8%) swabs were *K. pneumoniae*, *A. baumannii* (1.5% and 3.7%), *Enterobacter spp.* (0.5% and 0.4%) and *E. coli* (0.2% and 0.4%); while from nasal swabs 2% were MRSA. Finally, differences between group 1 and group 2 were assessed by isolated microorganisms, showing statistically significant differences for *Enterobacter spp*. (6% group 1 vs. 3% group 2; $p = 0.03$) and MRSA (13% group 1 vs. 5% group 2; $p = 0.0001$).
Discussion and Conclusions: Our data show an increase in screening swabs over the years. The trend, limited to the period of the pandemic, is different: there is an increase in MDR pathogens in COVID-19 patients assessed on all biological samples, with *A. baumannii* responsible for 68.7% of deaths from bloodstream infections. We demonstrated the importance of these swabs in the active surveillance of MDR bacteria and in improving infection control in hospital wards.

85 - Class-IIc bacteriocins produced by S. salivarius 24SMBc involved in antibacterial activity

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Categoria: Batteriologia, micologia e parassitologia

Titolo:
Class-IIc bacteriocins produced by *S. salivarius* 24SMBc involved in antibacterial activity

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Introduction: *Streptococcus salivarius* 24SMBc is an oral probiotic strain characterized by strong antimicrobial activity against potential pathogens that colonize the upper respiratory tract, like *Streptococcus pyogenes* and *Streptococcus pneumoniae*, and for this 24SMBc can be used in local bacteriotherapy to prevent *upper respiratory tract infections* (URTIs). Several clinical studies have reinforced the role of 24SMBc in reducing the recurrence of infection and rebalancing the nasal microbiota to a healthy status after antibiotic treatment. The aim of this work was to determine the presence of potential bacteriocins that could be responsible for the antimicrobial effect of this strain.
Materials and Methods: The genome of *S. salivarius* 24SMBc was sequenced with Illumina Miseq and de-novo assembled by SPAdes v3.14.0. Bioinformatic analysis with BAGEL4 and antiSMASH v 6.1.1. was performed to find bacteriocin genes carried by the 24SMBc genome.
Results: Genomic analysis of *S. salivarius* 24SMBc identified multiple genes involved in bacteriocin production and transport. BAGEL analysis found two genes encoding *Blp*-like class-IIc bacteriocins, *blpU* and *blpK*, in a cluster on contig 24. Moreover, antiSMASH confirmed the two bacteriocins genes in the same position and identified a peptidase_C39 encoding a putative ABC-type bacteriocin transport.
Discussion and Conclusions: These findings validate the presence of genes encoding for two different bacteriocins in 24SMBc genome, which could contribute to the antimicrobial activity exerted by this oral probiotic against other pathogenic streptococci, namely *S. pyogenes* and *S. pneumoniae*. Bacteriocin production is also important for the intra- and inter-species balance within microbial communities, and these results could further corroborate the efficacy of *S. salivarius* 24SMBc in rebalancing a healthy microbiota after local bacteriotherapy. In conclusion, the genome analysis confirmed the potential of this strain for its probiotic usage in clinical settings.

89 - Four-Year Environmental Surveillance Program of Legionella spp. in One of Palermo's Largest Hospitals

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Four-Year Environmental Surveillance Program of *Legionella* spp. in One of Palermo's Largest Hospitals

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Introduction: *Legionella* is a ubiquitous bacterium that lives in freshwater environments and colonizes human-made water systems. *Legionella pneumophila* is the most virulent species that may cause an atypical pneumonia after an incubation period of 2-10 days. Risk factors for disease include increasing age, smoking, chronic diseases and immunodeficiency. Because of this consideration, it is very important to monitor hospital water systems to prevent disease. Our laboratory, recognized as Regional Reference Center for Legionellosis in Western Sicily, according to ministerial guidelines, carries out surveillance in hospital buildings. We report the data achieved from the analysis of a four-year surveillance in a hospital of Palermo.
Materials and Methods: According to the standards of the Italian ministerial guidelines, we used the culture method, which is considered the gold standard for *Legionella* detection. The principal sites of sampling were, storage tanks, boilers (hot water), sinks and showers. Briefly, the samples were collected in sterile 1-liter containers, after filtration through a 0.2 µm cellulose membrane, 100 µL of the heat-treated sample and untreated sample was inoculated on two BCYE plate and incubated for 10 days at 37 ◦C in a incubator with 5% CO_2. The suspected colonies were confirmed using a latex agglutination test. The other species were identified by MALDI-TOF.
Results: Five areas were monitored over four years of time - Underground facility, Medicine, Surgery, Oncohematology, Others. A total of 251 samples were collected, 49% of which were *Legionella* spp. positive and 51% were negative. The percentages of positive samples for *Legionella* spp. collected during the period of study were very similar in all areas, with the exception of the Underground area. Positive samples with *L. pneumophila* sgr 2-15 were most frequent in the Medicine area. In addition, we identified 6/123 samples positive for *Legionella anisa.* Our date also shows how different strains coexist in the same sample.
Discussion and Conclusions: Legionnaires' disease could be a health risk for hospitalized patients, especially in immunocompromised patients. During these years of surveillance, it has been possible to detect a high bacterial load in the various areas that tends to be rather constant over time. Our data, based on the annual trend, show that the *L.pneumophila* sgr 2-15 strains are particularly resistant to water disinfection processes. The complete eradication of the germ is complicated for its ability to resist disinfection treatments, especially where it forms biofilms. In addition, the emergence of *L.anisa* increases the possibility of infection in hospitalized patients. Thus it is important to continue monitoring the water system to prevent legionellosis in patients.

94 - Occurrence of a KPC-3-producing Klebsiella michiganensis strain in clams

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Occurrence of a KPC-3-producing *Klebsiella michiganensis* strain in clams

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Introduction. Nosocomial infections caused by carbapenem resistant *Klebsiella michiganensis* have frequently been reported. Recently, *K. michiganensis* has also been isolated from wastewaters suggesting that faecal polluted coastal area may represent a potential source of human infections. This study describes the first recovery of a multidrug resistant (MDR) and KPC-3-producing *K. michiganensis* from Venus clams collected along the Adriatic Sea coast.
Materials and Methods. The *K. michiganensis* 23999A2 was selected from a clam sample plated on MacConkey agar supplemented with ertapenem. Carbapenem resistance was confirmed by the modified Hodge test, multiplex PCR for bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{OXA-48} and MIC determination. Plasmid profile and typing was determined by S1-nuclease pulsed field gel electrophoresis and PBRT 2.0 kit, respectively. The transferability of carbapenem resistance was investigated by conjugation experiments. Whole genome sequencing and a comparison with NCBI complete genomes was carried out. *K. michiganensis* pan-genome was also investigated.
Results. *K. michiganensis* 23999A2 was ertapenem resistant (MIC 256 µg/ml) and positive to the modified Hodge test. Carbapenem resistance was confirmed by the presence of KPC-3 encoding gene. The strain belonged to the new ST382 and carried seven plasmid replicon sequences including four IncF type plasmids (FII, FIIY, FIIk, FIB), one IncHI1 and two Col plasmids. FIB and FIIk replicons were found on plasmid contigs of ~49 kb and ~140 kb. The strain was positive for: bla_{OXA-9}, bla_{TEM-1A}, bla_{KPC-3} and bla_{SHV-12}, all plasmid-associated. The bla_{OXA-9} and bla_{TEM-1A} were carried by the FIB contig whereas bla_{KPC-3}, inserted in a Tn*4401a*, was carried by the FIIk contig. The bla_{SHV-12} was found on the FIIY plasmid. Conjugal transfer of both bla_{TEM-1A} and bla_{KPC-3} occurred at a frequency of 5×10^{-7}. The closest genome sequences to *K. michiganensis* 23999A2 were an environmental isolate collected from soil in South Korea and a clinical isolate sampled from human sputum in Japan. Of interest, *K. michiganensis* 23999A2 did not carry any bla_{OXY} gene. Pan-genome analysis revealed the presence of a large accessory genome.
Discussion and Conclusions.The report of an MDR KPC-producing *K. michiganensis* from Venus clam samples, and more globally from seafood product, pose a direct threat to public health. Filter-feeding molluscs can accumulate pathogenic bacteria and have a role in their transmission back to humans through the food chain and in the spread of antibiotic resistance in the environment. The great genomic plasticity of this strain highlights the capability of *K. michiganensis* species to adapt to new environments and underlines the risk of multidrug-resistant or hypervirulent clones emergence.

96 - In silico identification of novel inhibitors targeting the superoxide dismutase from the dental pathogen Streptococcus mutans and counteracting the biofilm formation.

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In silico identification of novel inhibitors targeting the superoxide dismutase from the dental pathogen *Streptococcus mutans* and counteracting the biofilm formation.

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INTRODUCTION - The microaerophile *Streptococcus mutans*, the main responsible for the development of dental plaque, has a single cambialistic superoxide dismutase (*Sm*SOD) for its protection against reactive oxygen species. In order to discover novel inhibitors of *Sm*SOD, possibly interfering with the biofilm formation by this pathogen, a virtual screening study was realised using the available 3D-structure of *Sm*SOD.
MATERIALS AND METHODS - Measurements of residual activity of *Sm*SOD in the presence of the putative novel inhibitors were performed through an indirect assay method. The oligomerization status of *Sm*SOD was analyzed by gel-filtration.
The possible toxic effect of selected molecules was investigated using the human fibroblast cell line BJ-5ta; total protein extracts obtained from BJ5-ta cells were used for Western blotting analysis.
The antibacterial activity of selected compounds against *S. mutans* was assayed by a standard broth micro-dilution method and the crystal violet staining method was used to measure biofilm biomass formed in the presence of the compounds.
RESULTS - Among the 36 molecules, compound ALS-31was capable of inhibiting *Sm*SOD with an IC_{50} value of 159 μM. Its inhibition power was affected by the Fe/Mn ratio in the active site of *Sm*SOD. Furthermore, ALS-31 also inhibited the activity of other SODs. Gel- filtration of *Sm*SOD in the presence of ALS-31 showed that the compound provoked the dissociation of the *Sm*SOD homodimer in two monomers, thus compromising the catalytic activity of the enzyme. Cell viability of the cell line BJ5-ta was not affected up to 100 μM ALS-31. A preliminary lead optimization program allowed the identification of one derivative, ALS-31-9, endowed with an improved inhibition power. Interestingly, below toxic threshold, planktonic growth and biofilm formation of *S. mutans* were inhibited by ALS-31, and even more by its derivativeALS-31-9.
DISCUSSION AND CONCLUSIONS - A deeper examination of the3D-structure of *Sm*SOD highlighted the possibility of targeting the dimer interface and disrupting the quaternary structure of this enzyme. Our results open the perspective of future drug design studies to fight dental caries, based on the identification of molecules acting on a crucial antioxidant enzyme target of *S. mutans*. Concerning the possible application of the identified novel molecules for treatment of dental caries, we can envisage their usage as a local topical medication, as the compounds have a direct antibiofilm activity on dental plaque formation by *S. mutans*.

98 - HPLC-MS-MS quantification of short-chain fatty acids secreted by probiotic strains

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HPLC-MS-MS quantification of short-chain fatty acids secreted by probiotic strains

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Introduction. Short-chain fatty acids (SCFAs) are the main by-products of microbial fermentations occurring in the human intestine and are directly involved in the host's physiological balance. As impaired gut concentrations of acetic, propionic, and butyric acids are often associated with systemic disorders in humans, the administration of SCFAs-producing microorganisms has been suggested as attractive approach to solve symptoms related to SCFAs deficiencies.
Materials and Methods. In this research, nine probiotic strains (*Bacillus clausii* NR, OC, SIN, and T, *Bacillus coagulans* ATCC 7050, *Bifidobacterium breve* DSM 16604, *Limosilactobacillus reuteri* DSM 17938, *Lacticaseibacillus rhamnosus* ATCC 53103, and *Saccharomyces boulardii* CNCM I-745) commonly included in commercial formulations were tested for their ability to secrete SCFAs by using an improved and sensitive protocol in high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS-MS).
Results. All tested microorganisms were shown to secrete acetic acid, with only *B. clausii* and *S. boulardii* additionally able to produce propionate and butyrate. Quantitative differences in the secretion of SCFAs were evidenced, thus demonstrating some strains being able to produce more acetic, propionic, and butyric acids than others.
Discussion and Conclusions. The application of a novel HPLC-MS-MS protocol for the detection and quantification of SCFAs allowed us to highlight species- and strain-specific behaviors for the *in vitro* synthesis and active secretion of SCFAs by probiotics. These data represent an innovation in the microbial characterization of strains commercialized in probiotic formulations, remarking the ability of only a few probiotic microorganisms present on the market to directly produce SCFAs. The direct production of SCFAs should be taken into consideration as a key beneficial feature when probiotic strains are evaluated for their clinical effectiveness, thus contributing to the spread of more targeted and personalized strategies to promote human health and manage diseases.

99 - IQOS vapours decrease Klebsiella pneumoniae growth without compromising lung cells compared to cigarette smoke

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IQOS vapours decrease *Klebsiella pneumoniae* growth without compromising lung cells compared to cigarette smoke

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Introduction: The use of Reduced-Risk Products (RRPs) is expanding rapidly around the world so an assessment of the biological activities of the vapors produced by RRPs is needed to delineate their properties after aerosolization. Nowadays, smoke reduction devices such as IQOS should be used instead of tobacco cigarettes with the commitment to reduce consumption over time until complete smoking and vape cessation. However, the question that most often grips is the possible increase in susceptibility to infections that these devices could cause. Anyway, to date few analyses have been carried out to get a precise representation of the effects of vapors on bacteria. Our study aimed to evaluate the antibacterial activity of aerosols produced by IQOS device. **Materials and Methods**: In order to evaluate the possible inhibitory activity against bacterial pathogens the IQOS Duo device equipped with Heets "Siena Selection" was chosen, the formula most requested by consumers. Moreover, the pathogen chosen for the test was the ATCC strain of *Klebsiella pneumoniae*, a known cause of pneumonia and chronic obstructive pulmonary disease exacerbations, which are also smoking related diseases**.** Besides, the bacterial reverse mutation test (Ames test), the primary component of the chemical safety assessment data required by regulatory agencies worldwide, was performed after exposure smoke/vapors generated from 1R6F cigarettes and IQOS, respectively, in order to detect mutagenic activities. Finally, human fibroblast lung diploid cells (MRC5) were exposed to cigarette smoke and aerosol from IQOS, to evaluate possible cytotoxic effects on lung tissue. **Results:** A very intense inhibitory activity was showed against *K. pneumoniae* by the vapors produced by Heets. Instead, no cytotoxic activity on lung cells or mutagenesis effects on TA98 - *S. typhimurium* tester strain was shown. In contrast, cigarette smoking caused evident damage to lung cells and showed a high rate of mutagenic capacity. **Discussion:** Our study proposed a standardized method for the evaluation of antimicrobial activity to be followed in this type of studies. Additionally, evidence of RRPs vapors effects on bacterial growth without compromising lung cells was demonstrated for the first time.

101 - Transcriptomics of VISA Emerging in a genomic context of CA-MRSA Under Teicoplanin Therapy

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Transcriptomics of VISA Emerging in a genomic context of CA-MRSA Under Teicoplanin Therapy

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Background

Outbreaks of Community-Acquired *Staphylococcus aureus* (CA- MRSA) infections have been reported worldwide supporting the increasing antimicrobial resistance trend also among the community circulating *S.aureus*. However, Vancomycin-Intermediate *S.aureus* (VISA) have been frequently reported among the health care-associated Methicillin Resistant *S.aureus* (HA-MRSA), whereas only few cases were recorded among CA-MRSA.

Methods

Our study aims to investigate the transcriptomic adaptations of a VISA strains clustering and genomically characterized as ST1-spatypet127-agrIII-SCCmecIVa-PVL-positive CA-MRSA, carrying the ant(6)-Ia and aph(3')-III (aminoglycoside-R), blaZ (β-lactam-R), mecA (β-lactam-R), ermC (macrolide-R) and tetK (tetracycline-R) acquired resistance genes, emerged in a patient under teicoplanin therapy. Comparative transcriptomics of the clinical VISA CA-MRSA versus its VSSA parent strain was investigated during the exponential growth-phase by RNA-Seq, Computational analysis was carried out by Rockhopper, bioinformatic, Enrichment and Gene-Ontology (GO) and KEGG.

Results

Transcriptomics provided insights into the complex nature of the transcriptomic adaptation traits of CA-MRSA strain versus its VSSA parent. Gene-Set Enrichment (GSE), Gene-Ontology and KEGG-pathway analysis evidenced a statistically significant dysregulation in sets of genes associated to different pathways. In particular, the over- and under-expressed gene clusters highlighted dysregulations in GO-term transcripts and pathway implicated in two-component regulatory systems, cell membrane structure, response to antimicrobials, oxidation and reduction processes, polysaccharide biosynthesis, protein/purine/amino sugar/nucleotide sugar metabolism, DNA-replication, transcription regulation and transport.

Conclusions

Our findings showed the complex nature of the VISA transcriptomic adaptations in the genomic context of a CA-MRSA superbug.

104 - Evaluation of the anti-biofilm activity of four different antibiotics against Staphylococcus aureus strains from ocular infections

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Evaluation of the anti-biofilm activity of four different antibiotics against *Staphylococcus aureus* strains from ocular infections

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Introduction: We investigated the biofilm forming ability of n=45 *Staphylococcus aureus* (14/45 MRSA; 31/45 MSSA) of the period 2017-2021 and involved in ocular infections, in Lombardy. We evaluated MICs/MBCs and Minimal Biofilm Eradication Concentration (MBEC) and the ability of levofloxacin (LEV), chloramphenicol (CAF), netilmicin (NET), and tobramycin (TOB) in avoiding cells adhesion and biofilm eradication. **Materials and Methods:** MICs/MBCs and a quantitative biofilm production were assessed for all the *S. aureus* isolates by broth microdilution (EUCAST breakpoints) and crystal violet assay, respectively. The biofilm inhibitory activity of the four antibiotics was tested on six strong biofilm producers selected as not clonally related by RAPD: 5/6 MSSA and the ATCC *S. aureus* 25923 strain using the crystal violet staining method. Biofilm biomass was quantified by staining with crystal violet and absorbance measurements at OD600nm. MBEC was performed by Calgary biofilm device. **Results:** 43/45 (95.6%) were CAF susceptible (93.5% MSSA *vs* 100% MRSA); 33/45 (73.3%) LEV susceptible (100% MSSA *vs* 14.3% MRSA); 28/45 (62.2%) resulted Wild Type (71% MSSA *vs* 42.9% MRSA); 32/45 (71.1%) TOB susceptible (100% MSSA *vs* 14.3% MRSA). All the *S. aureus* strains resulted strong biofilm producers. Regarding CAF, LEV, NET and TOB all the MSSA strains tested showed an CAF MIC=8mg/L and 0.125-0.25mg/L for LEV/NET/TOB (MBC= >32mg/L for CAF; 0.125-0.5mg/L for LEV; 0.125-0.25mg/L for NET and TOB); an enhancement in biofilm formation at the sub MIC values equal to 0.5mg/L (CAF); 0.03mg/L (LEV); 0.125mg/L (NET); 0.03 mg/L (TOB). The complete biofilm formation inhibition was near/at the MIC values of 8mg/L; 0.125mg/L; 0.25mg/L and 0.06, respectively. The MBEC results on cells organized in a 24h biofilm are: 512mg/L (CAF), 128-2048 mg/L (LEV), 256mg/L (NET); 32-64mg/L (TOB). **Discussion and Conclusions:** CAF/LEV/NET/TOB resulted effective in preventing bacterial adhesion and biofilm formation when used at concentrations equal to or higher than the MICs of the sensitive strains. Based on the dosages commonly reachable for CAF/LEV (with only one exception)/NET/TOB ophthalmic use, the results showed a clear efficacy in the eradication of newly formed biofilms of sensitive *S. aureus* strains. The efficacy of each drug against biofilm forming *S. aureus* strains is however subjected to the local epidemiology in terms of susceptible strains presence.

105 - Prevalence and virulence potential of Aeromonas spp. isolated from patients suffering from diarrhea

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Prevalence and virulence potential of Aeromonas spp. isolated from patients suffering from diarrhea

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Background - aim: Aeromonas spp are considered emerging human pathogens causing intestinal and extra-intestinal infections. In this study, we aimed to investigate the epidemiology of *Aeromonas* spp in patients with diarrhoea and characterize virulence mechanisms of human isolates.
Methods: During 14 months (5/2020 – 6/2021), 10856 diarrhoeic fecal samples were analyzed at the Microbiology Unit of Padua University Hospital using standard culture methods. *Aeromonas* spp were identified by sequencing the housekeeping gene *rpoB*. Antibiotic susceptibility was assessed by the VITEK-2 automated system, according to EUCAST criteria. The presence of genes involved in toxins coding such as *ela, ast, alt,* and *aer* was searched by polymerase chain reaction (PCR). Biofilms were grown in microtitre plates, stained with Crystal Violet, and the mean optical density (OD) quantified. Interaction of Aeromonas strains with Caco2 human intestinal epithelial cells (IEC) was assessed by quantifying adhesion, interleukin (IL)-8 secretion, integrity of epithelial barrier.
Results: 66 faecal samples were positive for Aeromonas spp (second most common enteropathogen identified): *A. caviae* (56,82%), *A. dhakensis* and *A. acquatica* (11,36) and *A. taiwanensis* (9%) were the most common specie. Infection was more common in subjects <10 years or >50 years old (32% and 59%, respectively). Forty-seven of *Aeromonas* isolates were resistant to amikacin whereas only 2.8% and 11,1% were resistant to ciprofloxacin or trimetoprim-sulfametoxazole, respectively. Multiple drug resistance was detected in 2 isolates, instead 30.5% strains were susceptible to all antibiotics. PCR analysis showed that all strains were *ela* (elastase) positive, whereas *ast, alt, aer* toxins were present in 57%, 66% and 45% of strains, respectively. Moreover, 25% of isolates reported the simultaneous expression of the three genes tested. Tested strains produced moderate biofilm (22,7% of strains had weak biofilm producing ability). *Aeromonas* isolates showed an excellent ability to interact with human IEC since: 43% of strains showed adhesion ability ≥1 CFU/cell; *Aer+* strains, as opposed to *aer-*, significantly reduced trans-epithelial resistance; 27% of isolates significantly enhanced release of interleukin-8, particularly *A. taiwanensis* strongly stimulate its production.
Conclusions: *Aeromonas spp* are relevant cause of diarrhea in humans, representing the second most common entero-pathogen isolated, affecting mainly young children and subjects older than 50 years. To date, human strains are still largely sensible to antibiotics. *Aeromonas spp* possesses a heterogeneous armamentarium of pathogenicity factors to efficiently interact with IEC, suggesting that *Aeromonas* spp induce diarrhea through different mechanisms.

106 - Spread of Metallo-Beta Lactamase genes: characteristics and diffusion of broad-host range and highly conjugative plasmids

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Spread of Metallo-Beta Lactamase genes: characteristics and diffusion of broad-host range and highly conjugative plasmids

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Introduction: The rapid spread of carbapenem resistant Enterobacterales is currently a worldwide growing public health threat. At the Policlinico Umberto I (PUI) of Rome, the surveillance performed in 2019–2021 on invasive infections showed that on 797 *K. pneumoniae* (*Kp*) isolates, 32% were carbapenem resistant. Most of these strains were KPC-producing strains. The high incidence of KPC-*Kp* is favouring the use of ceftazidime-avibactam (CZA), a novel beta-lactam/beta-lactamase inhibitor combination, as the first drug of choice for treatment of KPC-*Kp* infections. Metallo-Beta-Lactamases (MBL: New Delhi MBL, NDM; Verona integron-encoded MBL, VIM; imipenemase, IMP), confer resistance to both CZA and meropenem (MEM) and will increase their prevalence under selective pressure exerted by the intensive use of CZA treatment in the hospital. NDM in the last years reported an increment of its detection in Italy, due to the large outbreak occurred in Tuscany, while VIM was diffused at PUI accounting for 75.6% of all MBL-producing Enterobacterales in 2018.

Materials and Methods. Whole Genome Sequencing (Illumina and Nanopore technologies) was performed on VIM and NDM-producing strains from PUI hospital. VIM and NDM-carrying plasmids were compared with plasmids downloaded from a collection of 34,513 plasmids from GenBank (PLSDB database). We characterized the plasmids retrieved from VIM and NDM-positive strains by transformation in chemically competent *Escherichia coli* DH5 alpha cells.

Results: Analysis of data available at PUI on severe infections occurred in 2020-2021, showed that resistance to CZA combination is increasing in *Kp* strains from 4,0% to 6,2%. Among them the percentage of strains showing CZA and MEM co-resistance is also increasing going from 1% in 2020 to 2% in 2021. In 2020-2022, 18 strains were identified at PUI as NDM-producers (*Kp*, *Escherichia coli*, *Enterobacter cloacae*, *Providencia stuartii*), and 8 strains were positive for VIM (*Kp*, *E. coli*, *Klebsiella oxytoca*, *E. cloacae*, *Enterobacter aerogenes*). The bla_{NDM} genes were carried on different plasmid types, but the most frequent at PUI was the broad-host range IncC plasmid, identified in 83% of the NDM-positive strains in which bla_{NDM} was associated with the 16S rRNA methylase *rmtC*. The bla_{VIM} genes were carried on two major plasmid types of the IncN and IncA groups. The bla_{VIM}-gene cassette was typically inserted into integron platforms.

Discussion and conclusions: IncC and IncA are plasmids showing extremely wide host range and show high-efficient conjugation properties. By comparison of plasmids identified in different bacterial species throughout the years at PUI well demonstrated the ability of spreading. A multi-species bunch of VIM-1 and NDM-1 producing strains was identified in the hospital.

108 - Anatomy of pKpQIL, the blaKPC plasmid, and its role in ceftazidime-avibactam resistance

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Anatomy of pKpQIL, the *bla*$_{KPC}$ plasmid, and its role in ceftazidime-avibactam resistance

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Introduction. *Klebsiella pneumoniae* plays a crucial role in the global emergence of carbapenem resistance. In Italy this phenomenon is led by the spread of the plasmid-located *bla*$_{KPC}$ genes. The combination of ceftazidime-avibactam (CZA) has been introduced in clinical settings in 2018 for the treatment of serine-beta-lactamases producing bacteria. Yet, KPC-producing, CZA resistant *K. pneumoniae* isolates are emerging.

Materials and Methods. Whole Genome Sequencing (Illumina and Nanopore technologies) was performed on 23 KPC-producing CZA-resistant strains from the Policlinico Umberto I (PUI) hospital. Most of them (18) carried *bla*$_{KPC}$ on a pKpQIL plasmid. These plasmids were compared with 104 plasmids carrying the FIB(pQIL) and FII(K) replicons downloaded from a collection of 34,513 plasmids (PLSDB database). Statistical (chi-squared, Pearson's and Spearman's R) and bioinformatic (pangenome definition, phylogenesis, detection of associating genes, estimation of average nucleotide identity) analyses were carried out on data from the 122 genomes. Furthermore, we characterized the activity against CZA of the KPC-producing *K. pneumoniae* isolates retrieved from PUI by transformation of the respective pKpQIL plasmids in chemically competent *Escherichia coli* DH5-alpha cells.

Results. pKpQIL plasmids have a length ranging from 85,040 to 307,743 bp (median 116,381, IQR 21,363 bp) and a GC content ranging from 50.705 to 54.783% (median 53.663, IQR 1.209). The typical pKpQIL plasmid carries few resistance genes (mode and median = 2 genes), in most cases KPC (83/122, $p< 0.00001$) or other beta-lactamases (NDM-1 in 8 cases and CTX-M-15 in 14 cases) but they can harbor up to 16 different resistance genes. As these plasmids grow in length, their GC content decreases ($R=-0.509$, $p=0.00002$) and they have more resistance genes ($R=0.653$, $p<0.00001$). The pangenome of pKpQIL plasmids is made up of 1,739 genes, 24 of which are present in more than 90% of the genomes (i.e. the plasmid core genome).

CZA-resistant isolates harboring *bla*$_{KPC}$ on a pKpQIL-like plasmid from PUI belonged to three high-risk clones: ST512, ST37 and ST307. MICs for CZA ranged from 8,0 mg/L to >256 mg/L and were mediated by 8 different KPC-variants. In 5 cases co-resistance to meropenem was observed. *E. coli* transformed with pKpQIL displayed comparable characteristics.

Discussion and Conclusions. While KPC-31 (D179Y variant of KPC-3) has been described as one of the major causes of CZA resistance in KPC-producing *K. pneumoniae* worldwide, KPC-66, -67, -69, -70 and -110 have been isolated for the first time in PUI. These data suggest that the spread of *bla*$_{KPC}$ is related to pKpQIL and that, in high endemicity settings, CZA-resistance is driven by the pliability of the *bla*$_{KPC}$ gene in a subset of high-risk clones.

109 - A new core-genome based typing method for Providencia stuartii: characterization of New Delhi Metallo-Beta-Lactamase-producing strains causing an outbreak

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A new core-genome based typing method for *Providencia stuartii*: characterization of New Delhi Metallo-Beta-Lactamase-producing strains causing an outbreak

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INTRODUCTION:

Providencia stuartii is a gram negative, highly mobile, opportunistic pathogen member of Enterobacterales. *P. stuartii* causes urinary and respiratory infections in nosocomial contest. It shows intrinsic resistances to the polimixyn and carries intrinsic genes like *tet(B)*, *catA3* and *aac(2')-Ia*.-*P. stuartii* typing has not yet been implemented and it doesn't exist a multi locus sequences typing (MLST) scheme for this opportunistic pathogen. Here, we describe a 4-patient outbreak occurred in two intensive care units (ICUs) of the Policlinico Umberto I (PUI) caused by NDM-producing *P. stuartii*. To trace *P. stuartii* of this outbreak and compare them with those circulating worldwide, we established a new MLST scheme based on seven genes and a core genome based MLST

MATERIALS AND METHODS:

Seven NDM-producing *P. stuartii* strains from four patients were identified in three months at the PUI hospital. Complete genomes were obtained for 3 prototypic strains by Illumina MiSeq and Oxford Nanopore Technologies. Genomes were assembled by Unicycler and analysed by Prokka for gene assignment and Staramr for identification of antibiotic resistance and plasmid content. IncC complete plasmid sequences were compared with related plasmids previously identified at the PUI. CHubbaca and other tools were utilized for identification of *P. stuartii* core genome to build up effective MLST and core genome MLST. The novel MLST method was applied to PUI isolates compared with 54 *P. stuartii* genomes downloaded from Genbank.

RESULTS:

Analysis on the *P.stuartii* genomics from PUI showed high phylogenetic similarity of strains causing the outbreak, with a range of 0-2 SNPs on the core genome. We identified two plasmids carried by *P. stuartii* strains: one ColpVC present only in 1/3 strains and an IncC plasmid detected in the 3 isolates analysed. The IncC plasmid carried numerous resistance genes, including bla_{NDM}, bla_{CMY-6} and the 16S rRNA methyltransferase *rmtC* and conferred resistance to all beta-lactams and aminoglycosides. It was indistinguishable from the IncC causing an outbreak in NDM-producing *Klebsiella pneumoniae* ST15 clone occurred in 2020 at PUI.

DISCUSSION AND CONCLUSION:

The IncC plasmid previously observed in ST15 *K. pneumoniae* causing an outbreak in same hospital, was unseen for approximately 2 years and then recurred in *P. stuartii.* Low differences between these plasmids were identified: *K.*

pneumoniae IncC showed one IS3 that was lost in the IncC from *P.stuartii* that acquired one IS1 and a *K. pneumoniae* genome fragment. This acquisition traced the origin of the plasmid from *K. pneumoniae*. We used genomic data to build up pangenome and coregenome, establishing a global typing method for the *P. stuartii* species, currently divided in 21 different phylogenetic groups.

112 - Study of Acinetobacter species resistance patterns and virulence in a multidisciplinary hospital

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Study of *Acinetobacter* species resistance patterns and virulence in a multidisciplinary hospital

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1. Introduction: *Acinetobacter* genus includes aerobic, non-fermenting, gram negative coccoid rods. Their extensive survival on various surface of medical equipment in hospital units is alarming. Each year, the number of human infections caused by these conditional pathogens is rapidly increasing, therefore WHO labeled *Acinetobacter spp.* as "critical level or priority". They can hold responsibility of serious infections especially in debilitated patients that undergo invasive medical procedures. Antibiotic resistance profile shows a multi-drug-resistance pattern. The aim of the study is to evaluate the dynamics of *Acinetobacter* infections spectrum and to assess the resistance patterns of *Acinetobacter spp*. identified from hospitalized patients' samples in a multidisciplinary hospital. **2: Materials and Methods:** This is a retrospective descriptive study that includes 1164 *Acinetobacter* strains isolated from patient samples from Clinical County Emergency Hospital of Brasov during 1st of January 2018-31st of December 2021. Data was collected from MEDIS system and WHO-NET database provided by Clinical County Emergency Hospital of Brasov. Data was processed in Microsoft Excel Worksheet. Bacterial identification was performed based on biochemical testing. Antimicrobial susceptibility of microorganisms was evaluated by means of disk diffusion method and confirmed by automated VITEK 2 COMPACT. **3. Results**: *Acinetobacter spp.* have been frequently identified in patient samples from intensive care units (53.4%) and plastic surgery department (11%). Most samples were respiratory secretions (38%), wound secretions (26%) and pus (18.5%). More than 80% of tested strains were resistance to both Imipenem and Meropenem. **4. Discussions and Conclusions:** This study underlines the importance of constantly monitoring the antibiotic resistance pattern of *Acinetobacter spp.* pathogens with the aim of optimizing the treatment prescription, especially regarding the empirical antimicrobial therapy.

117 - Activity of cell-free supernatants from probiotic Lactobacillus strains against Pseudomonas aeruginosa from cystic fibrosis patients

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Activity of cell-free supernatants from probiotic *Lactobacillus* strains against *Pseudomonas aeruginosa* from cystic fibrosis patients

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Introduction: The potential application of probiotics is continuously widening, although the therapeutic use of live bacterial cells may pose safety concerns, especially in vulnerable subjects. New evidence points to postbiotics as a step forward in the use of pre- and probiotics. Roughly defined as a complex mixture of metabolic products secreted by probiotics in cell-free supernatants (CFS), postbiotics may have several advantages over probiotics, including safety, ease of production and storage, and multiple mechanisms of action. As part of a project funded by the Italian cystic fibrosis (CF) foundation, in this study we evaluated the antibacterial, antibiofilm, and anti-virulence properties of CFS from *Lactobacillus* strains against *Pseudomonas aeruginosa* isolated from CF patients.

Materials and Methods: CFS were prepared by growing *Lactobacillus* strains in MRS broth for 48 h at 37°C in agitation, followed by centrifugation of the cultures and filtering of the supernatants. Growth inhibitory activity was assessed against mucoid and non-mucoid strains via the agar-well diffusion method and by monitoring the optical density over 24 h of incubation. To mimic the CF lung environment, killing kinetics against planktonic bacteria and mature biofilms were performed by exposing *P. aeruginosa* strains to CSF in an artificial sputum medium (ASM) and evaluating CFU counts at different time intervals.

Results: CFS (pH approximately 4.0) from multiple *Lactobacillus* strains exerted marked growth inhibitory and killing capacity towards several *P. aeruginosa* strains, with CFS from *L. rhamnosus* and *L. plantarum* being the most active. Such capacity was abolished when the pH was adjusted to 6.0 with NaOH. Nevertheless, CFS exerted higher inhibitory capacity and killing activity than sterile MRS broth adjusted at pH 4.0 with HCl, suggesting that the inhibitory capacity was not merely due to the acidity. CSF also exerted a fast and strong antibacterial and antibiofilm activity in ASM. The evaluation of the anti-virulence properties of CFS is ongoing.

Discussion and conclusions: The use of postbiotics, a term recently revised to include both metabolites and fragments derived from microorganisms, seems an attractive therapeutic/preventive strategy in the era of antibiotic resistance. Herein we demonstrated their activity towards clinical strains of *P. aeruginosa* in conditions mimicking CF lung. Future studies are warranted to identify the full spectrum of such metabolites' activities and unveil innovative postbiotics' uses in anti-infective therapy. *The study received support from the Italian Cystic fibrosis research foundation, Project FFC#13/2021.*

120 - Antibiofilm activity of Moringa oleifera Lam. leaf extracts by Scanned Electron Microscopy visualization against Xanthomonas campestris pv. campestris

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Antibiofilm activity of *Moringa oleifera* Lam. leaf extracts by Scanned Electron Microscopy visualization against *Xanthomonas campestris* pv. *campestris*

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Introduction *Xanthomonas campestris pv. campestris* (XCC) is the causal agent of black rot in crucifers, a plant disease with high economic impact. Xanthomonodaceae are a large genus of Gram-negative bacteria that cause their symptoms by blocking plants water flow by invading the xylem. To accomplish that, biofilm formation is the main mechanism that XCC uses to adapt to environmental changes and to colonize tissues. In recent years, the growing interest in natural antimicrobial compounds led to the study of different phytocomplexes derived from different plants. In our project, *Moringa oleifera* Lam. (MOL), was selected, as MOL leaves are rich in phenols, essential oils, and vitamins that exert antibacterial properties. Biofilms are often studied with scanned electron microscopy (SEM), and therefore XCC biofilm formation and removal were analyzed on abiotic and biotic surfaces, with and without the influence of different MOL leaf extracts.

Materials and methods Biofilm formation and removal assay: XCC strains, isolated from the Emilia-Romagna phytosanitary department, were inoculated in LB broth with MOLs at non-lethal concentration in a 6-well plate containing a cell strainer for 120 h at 25 °C. For biofilm removal, after incubation and after visually assessing biofilm formation, MOLs at non-lethal concentration were added and incubated for 48 hours.

In planta biofilm formation: XCC suspensions, containing 10^6 CFU/mL, were inoculated in LB broth in a 4 weeks old cabbage, and then incubated for 7 days at RT. Once the infection started, MOLs at their MIC were added.

Biofilm SEM visualization: samples were fixed in 2.5% glutaraldehyde in 0.1M KPO_4 buffer, dehydrated in alcohol and dried with critical point dryer. Samples were mounted on metal stubs and gold-coated. Samples were examined with SEM Zeiss Evo40.

Results The assay showed a complete colonization by XCC on abiotic and biotic surfaces if not under the effects of MOLs: the control, in fact, showed that the fibers of the cell-strainer were coated with EPS, as well as cabbage xylem was completely invaded; furthermore, the production of xanthan gum (one of the main components of XCC EPS and virulence factors) laminas was observed. The fact that HAMD-MOL and MeOH-MOL can exert antibiofilm properties is proven and confirmed by the presence of just few solitary planktonic bacteria, and no complex or organized structures were observed.

Conclusions In conclusion, XCC can adhere to both abiotic and biotic surfaces developing its biofilm on them, while, when under the effects of MOLs, XCC changes its cell shape and interrupts that ultrastructural organization that characterize a developed biofilm, in particular in the adhesion part of the process, and this may have important applications in disease control.

121 - Effects on Xanthomonas campestris pv. campestris by Moringa oleifera Lam. leaf fermented extracts

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Effects on *Xanthomonas campestris pv. campestris* by *Moringa oleifera* Lam. leaf fermented extracts

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Introduction *Xanthomonas campestris pv. campestris* (Xcc) is the causal agent of black rot in crucifers, a plant disease with high economic impact. Biofilm formation is the main mechanism that Xcc uses to adapt to environmental changes and to colonize tissues. In recent years, the growing interest in natural antimicrobial compounds led to the study of different phytocomplexes with antimicrobial properties derived from the vegetable world. For this reason, *Moringa oleifera* Lam. leaves (MOL) were selected and studied by our group. After the extraction processes, waste material can still be rich in active substances: in this project we focused on an Herbal Medicines By-product generated from the extraction processing of MOL leaves (MHMB) and on its antimicrobial effects.

Materials and methods MHMB have been suitably diluted and submerged fermentations with 5 different species of fungi have been carried out for 35 days under optimal stirring and temperature conditions. Minimal Inhibitory Concentration (MIC) was assessed. Biofilm formation and removal assay: XCC strains, isolated from the Emilia-Romagna phytosanitary department, were inoculated in LB broth with MOLs at non-lethal concentration in a 96-well plate 72 h at 25 °C. For biofilm removal, MHMB at non-lethal concentration were added after incubation and after visually assessing biofilm formation and incubated for 48 hours. The crystal violet (CV) method was then used. Membrane permeability (MP) was assessed by the propidium iodide assay. MP was examined after 30, 60, 120, 180 minutes of contact with the different MHMB.

Results and conclusions From all the different MHMB tested, only one of the fermented extracts could significantly alter membrane permeability and biofilm formation processes in a *in field*-usable concentration. In fact, MHMB-PC could alter XCC membrane permeability already after 10 minutes of contact at a [V/V] concentration of 12.5%. Regarding its antibiofilm activity, the removal results appear to be promising, leading to the removal of 87% of a mature biofilm when used at a sub-lethal concentration.

122 - A fast microbiology experience in a Southern Italy hospital: a diagnostic stewardship approach.

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A fast microbiology experience in a Southern Italy hospital: a diagnostic stewardship approach.

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Introduction

Diagnostic microbiology has remained relatively unchanged for decades, but recent years have witnessed a remarkable effort to develop novel technologies for faster microbiological diagnosis of sepsis. Faster identification of blood culture pathogens was associated with a shorter time for adequate therapy and decreased mortality in combination with antibiotic stewardship programs (ASP). The diagnostic stewardship is part of a unified stewardship strategy and means: right patients, appropriate test, right interpretation of the results at the right time. We proposed in laboratory routines one of rapid diagnostic test to improve BSI management. The study aims to emphasize advantages in using this fast panel to perform a BSI diagnosis with a complete rapid susceptibility profile.

Materials and Methods

The study involved 11 positive blood cultures from septic patients at Sant 'Anna and San Sebastiano Hospital of Caserta. None of patients underwent empiric treatment. Blood samples were collected and flagged positive within a 7-hour medium incubation time in a continuously monitored system. Identification and antimicrobial susceptibility tests were performed according to the manufacturer's instructions within 8 hours in Accelerate *Pheno*, with a medium time length of respectively 1 hour and 30 minutes and 6 hours and 30 minutes.

Result

5 *Acinetobacter baumannii* and 4 *Klebsiella pneumoniae*, 1 *Proteus mirabilis* and 1 *Serratia marcescens* were isolated. For all the gram negative identificated MIC value for the principal antibiotic were obtained.

Particularly colistin MIC for *A. baumannii;* 3dg cephalosporins and carbapenem for *Klebsiella spp.* and MIC values for amikacin, trimethoprim/sulfametoxazole, piperacillin/tazobactam were obtained for *Serratia* and *Proteus*. The test also performed for all the strains the antimicrobial drug for Multidrug Resistance Organism like Ceftazidime/Avibactam and Ceftolozane/Tazobactam. Thanks to the availability of a fast susceptibility profile a definitive therapy was prescribed to the patients. BSI had different curses: all the *A. baumannii* were resistant to all the tested antibiotic except for colistin. The *Klebsiella pneumoniae* showed carbapenem resistant profile (1 OXA-48 and 4 KPC) and were treatment with ceftazidime/avibactam. *Serratia* had susceptibility for all the tested antibiotics.

Discussion and conclusion

The future of sepsis therapy has led to many attempts to personalize the management of sepsis patients involving fast microbiology diagnostic technologies like Accelerate *Pheno* test. Rapid identification of the infection and its antibiotics sensitivity is of primary importance for improving the turnaround time in BSI but also in the infection control program. In this way a unified strategy integrated the diagnostic stewardship improve the management of sepsis and the clinical outcome.

125 - A case report of oral candidiasis in an immunocompetent patient

Case report: Disseminated cryptococcus neoformans in an immunocompetent patient.

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Introduction: Cryptococcosis is an opportunistic fungal disease, caused by *Cryptococcus grubii*, *Cryptococcus neoformans* (*C. neoformans)*, and *Cryptococcus gattii*. These species mainly lead to lung infections and central nervous system infections, such as meningoencephalitis and cranial neuropathies. Other types of infections include skin, prostate, eyes, bone marrow and joints. Infection occurs more easily in patients with immunosuppression or with other infections. A very high mortality rate was reported for cryptococcal meningitis ranging from 27% to nearly 50%. We describe one case of meningoencephalitis from Cryptococcus neoformans that occurred in an apparently immunocompetent patient.

Materials and Methods: A 38-year-old Ukrainian woman was reported to be in excellent health up to 2 weeks before hospitalization, when she developed fever and confusion. After 48 hours, progressed to visual hallucinations along with urinary incontinence. The differential diagnosis included pulmonary tuberculosis, bacterial or fungal pneumonia, and lung cancer. The patient was subsequently diagnosed with disseminated *C. neoformans*, which remains very rare.
Results: A diagnosis of bacterial meningitis was made, and ceftriaxone and corticosteroids were given to reduce inflammation and edema of the brain and cranial nerves. Two days later, due to lack of clinical improvement, an aliquot of the cerebrospinal fluid (CSF) was tested, and it was found that she had a cryptococcal antigen title of 1: 320. Within 72 hours of culture, the laboratory notified doctors that *C. neoformans* was isolated from CSF. Using mass spectrometry (MALDI-TOF MS), the isolate was identified as subspecies *C. neoformans.* Therapy was changed to amphotericin B and her mental status improved significantly over the next 1 week and her cerebrospinal fluid culture had a decreased CSF cryptococcal antigen title to 1: 160.
Discussion and Conclusions: *C. neoformans* have a complex polysaccharide capsule with antiphagocytic properties. Low concentrations of anticryptococcal antibodies are normally found in immunocompetent people due to daily exposure. Although exposure is almost omnipresent in some regions of the world, this organism rarely causes clinically important infections in immunocompetent hosts. Conversely, it has become a notable opportunistic infection in those possessing a compromised cell-mediated immune response. Our case highlights the importance of collecting an accurate travel history in all patients, as the differential diagnosis should include atypical pathogens that may be endemic to the travel area. It also highlights the significant morbidity associated with cryptococcosis and drug-related toxicities and methods of preventing complications.

126 - Comparison of Loop Mediated Amplification (LAMP) and quantitative Real Time PCR (qRT-PCR) methods for the routine detection of Pneumocystis jirovecii.

Neill Adams [(1)] - Nadia Marascio [(1)] - Aida Giancotti [(1)] - Marta Pantanella [(1)] - Giuseppe De Angelis [(1)] - Mariangela Cassadonte [(1)] - Michele Manno [(1)] - Grazia Pavia [(1)] - Francesca Divenuto [(1)] - Angela Quirino [(1)] - Giovanni Matera [(1)]

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Comparison of Loop Mediated Amplification (LAMP) and quantitative Real Time PCR (qRT-PCR) methods for the routine detection of *Pneumocystis jirovecii.*

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Introduction

Pneumocystis jirovecii is the causative agent of Pneumocystis Pneumonia (PCP) in immunocompromised hosts. HIV positive patients demonstrate higher fungal loads and less severe clinical course than non-HIV patients. *Pneumocystis* is species specific and healthy humans represent an important reservoir. Routine diagnosis requires identification of *Pneumocystis* forms in respiratory samples by microscopy or nucleic acid detection by molecular assays. The measurement of 1,3-beta-D-glucan (BDG), is not specific for *Pneumocystis*, but has a high negative predictive value for PCP. This study aimed to compare traditional microscopy with commercial Loop-mediated isothermal Amplification (LAMP) and quantitative Real Time-PCR (q RT-PCR) methods.

Materials and Methods

Between January 2019 and June 2022, 180 respiratory samples from 132 patients were analyzed by microscopy during routine screening for *Pneumocystis.* Retrospectively, microscopy positive or microscopy negative/BDG positive samples were analyzed using molecular methods. Microscopy was performed by Giemsa staining (RAL 555, RAL Diagnostics) and direct Immunofluorescence, (Mono-fluo *P. jirovecii* IFA test kit, Biorad). BDG was tested using kinetic turbidometry (ALIFAX). LAMP and qRT-PCR were performed with EASYplex® (Amplex Diagnostics GmbH) and Realstar® *Pneumocystis jirovecii* kit 1.0 (Altona Diagnostics GmbH), respectively.

Results

We selected thirty-six patients based on positivity with microscopy (n=9, male 88%, age 32-88, mean 59.6) or microscopy negative but BDG positive (n=27, male74%, age 19-83, mean 59.9) Utilizing the LAMP method, 4/36 (11.1%) patients were found positive, whilst 7/36 (19.4%) were positive with qRT-PCR, with fungal loads varying from 1 to 1,24 x 10^6 copies/ml. All LAMP positive results were confirmed by qRT-PCR. In 4/9 (44%) microscopy positive patients, results were not confirmed by either molecular method. In 4 patients with negative microscopy but positive BDG, LAMP was negative. However, the qRT-PCR was positive, but with low fungal loads (10,2 – 110 copies/ml). In particular, 25/27 microscopy negative/ BDG positive patients were co-infected with other fungi (*Candida spp* or *Aspergillus spp*).

Discussion and Conclusion

Microscopy is susceptible to false positive and negative results and may require other confirmatory tests. The Easyplex LAMP assay (limit of detection around 3000 copies/ml) is time-saving, easy to perform and a positive result may be associated with PCP diagnosis. The qRT-PCR is a highly sensitive method, but a positive result requires careful interpretation in parallel with clinical data to separate PCP patients from colonized patients.

130 - INCREASE OF KPC-PRODUCING KLEBSIELLA PNEUMONIAE IN A NEONATAL INTENSIVE CARE UNIT: A THREE- YEARS EVALUATION

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INCREASE OF KPC-PRODUCING KLEBSIELLA PNEUMONIAE IN A NEONATAL INTENSIVE CARE UNIT: A THREE- YEARS EVALUATION

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INTRODUCTION: Carbapenem-resistant *Klebsiella pneumoniae* (KPC) infections represent a critical issue worldwide and also a challenge in medicine, due to therapeutic difficulties and high mortality rates. Italy is KPC endemic and a significant KPC increase strains has been reported since 2010. Nowadays, KPC is considered the most common cause of hospital-acquired pneumonia, especially in ICUs, including NICUs. Active surveillance protocols are considered the most effective strategy to prevent invasive infections, especially in the case of patients actively colonized by multidrug-resistant (MDR) Gram-negative. A three-year (2019-2021) statistical evaluation was performed considering NICU from University Hospital Policlinico of Catania.

MATERIALS AND METHODS: We analyzed microbiological data from NICU samples collected between 2019 and 2021, considering potential changes in microbial colonization or infection. Collected samples were related to surveillance controls, performed through oro-pharyngeal, nasal and rectal swabs, and also respiratory and urinary samples. Gram-negatives percentages were compared along the three years, with particular regard to KPC isolates.

RESULTS: Among 2019 isolates, only 6.25% were positive for KPC, while 37.50% showed complete antimicrobial susceptibility and 56.25% produced extended-spectrum beta-lactamases (ESBL). Considering *K. pneumoniae* positive cultures, 2020 revealed a KPC isolation rate of 34.14%. ESBL isolates were equal to 9.70%, while 56.01% were completely susceptible to antibiotics. 2021 KPC isolates hold a percentage of 63.90%. In the same year, 2.8% were ESBL producers, while 33.40% showed completely antimicrobial susceptibility.

DISCUSSION AND CONCLUSION: *K. pneumoniae* can be considered one of the most common aetiological agents of nosocomial infections. Critically ill patients are significantly exposed to its colonization. Among this type of patient, newborns seem to be particularly prone to develop invasive infections, due to a possible preterm birth and an incomplete structuring of the immunological system. According to our data, KPC percentages progressively increased from 2019 to 2020, with a significantly high spread during 2021. These results highlight the importance to intensify sanitizing and surveillance protocols in intensive care units, with particular regard to NICUs. Protocols should implement hygiene education programs and eventual patient isolation, due to reduce bacterial transmission and diffusion.

131 - Human infection by Trichostrongylus spp. in an immigrant patient living in an asylum seekers center in Southern Italy

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Human infection by *Trichostrongylus* spp. in an immigrant patient living in an asylum seekers center in Southern Italy

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Introduction: Nematodes of the genus *Trichostrongylus* are ubiquitous parasites found in the digestive tract or respiratory system of several animals (widely spread among the herbivores), with a global distribution. Sheep and goats are important reservoirs of these parasites, but chickens also can be infected. The life cycle takes place when eggs are passed in the feces of infected animals. Subsequently, first-stage larvae (L1) hatch from mature eggs and develop into the second stage (L2) and then into the third stage (L3) infective larvae. The transmission of the parasite to humans is often related to the ingestion of water or unwashed vegetables contaminated with infective larvae. Although the prevalence of infections is low and usually asymptomatic, in the most severe cases, the hematophagism of the infective larves can cause inflammation of the bowel mucosa and tissue damage with clinical symptoms as cramping, abdominal pain, flatulence, nausea and diarrhea.
Materials and Methods: From February 2022, n. 50 patients coming from different region of Sub-Saharian Africa and temporarily hospitalized in an asylum seekers center (Borgo Mezzanone, Foggia, Apulia Region) were screened for the presence of gastrointestinal parasites. From each individual, a fecal sample was collected and subjected to microscopic investigation by using the Mini-FLOTAC® technique. Data referencing age, gender, nationality, physical complaints and other microbiological analysis were collected from enrolled patient laboratory records.
Results: Out of 50 fecal samples subjected to coprological screening, n. 1 (1/50, 2%) was positive to gastrointestinal parasites and eggs of *Trichostrongylus* were detected by microscopic examination. No other eggs/cystis/oocystis related to other parasites were found in the sample. The infected patient was a man of 21 years old coming from Burkina Faso.
Discussion and Conclusions: This is the first report of a case of *Trichostrongylus* infection in an immigrant patient coming from Africa and temporarily living in Italy. Incidence and prevalence of *Trichostrongylus* spp. infections change significantly depending on population and geographical region studied, with rates of prevalence higher in rural areas. The direct contact with animals, the water reservoirs for human consumption contaminated with feces of ruminants, rodents or even humans, water used for irrigation contaminated with infective larvae and the poor socioeconomic conditions and hygiene may represent risk factors for the infection. The source of *Trichostrongylus* infection in this patient is uncertain. Further studies on the *Trichostrongylus* spp. prevalence in human hosts should be carried out in order to adopt specific measures to control widespread parasitic infections.

136 - Bacteria from the vaginal microbiota cause suppurative hysteritis/pelvic inflammation in mice treated with estrogens

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Bacteria from the vaginal microbiota cause suppurative hysteritis/pelvic inflammation in mice treated with estrogens

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Introduction: The health status of the female genital tract is largely related to the presence of a normal vaginal microbiota. It is known that estrogens influence the vaginal environment, affecting the quality and quantity of resident microorganisms. The vaginal microbiota is indeed modified by hormonal and physiological changes that occur during the menstrual cycle, pregnancy and in menopause, leading to microbial 'dysbiosis', including bacterial vaginosis and aerobic vaginitis. In both diseased the normal vaginal microbiota shifts to a population dominated by enterobacteria, staphylococci, streptococci, enterococci or anaerobic microorganisms. In the present work we report the setup of a mouse model of genital tract infection by bacteria of the indigenous microbiota following estrogen treatment.
Materials and Methods: C57BL/6 mice were subcutaneously treated with 100 μg of 17-*beta*-estradiol and the changes in the normal vaginal flora were characterized 7, 14, 21 and 28 days following hormone treatment by standard microbiological and molecular techniques. The establishment of the upper genital tract infection was confirmed by cultural analysis of suppurative material collected in the uterus and by histological examination of samples collected at the sacrifice.
Results: In this study, a pelvic inflammation was observed in mice after estrogen treatment, with bacteria such as *Staphilococcus aureus, Proteus mirabilis, Klebsiella aerogenes* and *Enterococcus faecalis* isolated in vaginal swabs and from the suppurative material collected in the uterus of the same mice. The genital tract infection caused by indigenous microbiota was confirmed by histological examination that showed the uterine cavity completely filled with suppurative material (pyometra).
Discussion and Conclusions: This murine model of infection is an optimal tool in the study the complexity of the genital tract infections, to discover biomarkers of health/inflammation and furthermore to evaluate new antimicrobial agents aimed at guaranteeing the well-being of women and reducing the risks associated with any obstetric complications.

138 - Antibiofilm activity of Ciprofloxacin-loaded niosomes.

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Antibiofilm activity of Ciprofloxacin-loaded niosomes

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Introduction. Antimicrobial resistance is one of the main problems of Public Health with important clinical and economic implications. A phenomenon that leads to an increase in antibiotic resistance is linked to the ability of bacteria to form biofilm, a microbial community immersed in an exopolysaccharide matrix in which bacterial cells are protected from the action of the immune system, disinfectants, and antibiotics. Ciprofloxacin (CIP), that belongs to the class of fluoroquinolones, has been used extensively against various bacterial infections; however, an increasing proportion of clinical isolates have proven resistant. To improve the bioavailability and the effectiveness of antibiotics, even against bacterial strains producing biofilm, the use of nanotechnology could play a fundamental role. CIP transport studies with nanocarriers have shown reduce side effects, increase stability, release control and decrease antibiotic resistance. In this work, the activity of niosome preparations, loaded with CIP, were tested against Gram-positive and Gram-negative bacterial strains.

Materials and Methods. A deep physical-chemical characterization in term of stability over time/biological media and drug entrapment efficiency was carried out on niosome preparations. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of these sample was estimated by the broth micro-dilution method against Gram-negative and Gram-positive strains. To measure the biofilm inhibition induced by CIP-loaded niosomes, the growth medium was supplemented with sub-MIC concentrations of nanocarriers. To evaluate the uptake of niosome formulations into epithelial cultured cells, CIP and Nile Red were loaded inside the nanocarriers. The cytotoxicity was determined on cells monolayers by MTT assay.

Results. All the samples presented nanometric dimensions and a good stability profile. The CIP-loaded niosomes exhibited an antibacterial activity against all tested strains, in a dose dependent manner, but lower respect to free CIP. Furthermore, when delivered in niosomes, CIP showed a greater ability to inhibit biofilm formation against strong biofilm producer strains, compared to free CIP. The internalization of niosomes occurred in cells monolayers already after 7 hs of contact. The cytotoxic effect of these preparations was detectable only at the highest concentrations.

Discussion and Conclusions. It has been reported that niosomal vesicles, due to their cationicity, interact electrostatically with the negatively charged biofilms; the drug can be released into the biofilm structure. Our preliminary results confirmed that the delivery of CIP by nanocarriers may be very promising to inhibit biofilm formation by both Gram positive and Gram negative bacteria.

144 - Cryptococcal Meningitis

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Cryptococcal Meningitis

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Introduction: Cryptococcosis is an opportunistic infection, caused by *Cryptococcus spp*., that lives in the environment throughout the world. Even though the infection is usually HIV-related, most of the non-HIV-related cases include patients under immunosuppressive treatments or with organic failure syndromes. The organism is acquired by inhalation. While all organs can be involved, *Cryptococcus spp*. have a strong affinity for the central nervous system. Cryptococcal meningitis usually presents as a subacute meningoencephalitis.
Materials and Methods: The company operating procedures provide for the execution of at least
2 portions of CSF, each of at least 1ml. The first rate is used for the physical and chemical examination. The second rate is used for microbiological tests: crops before and after enrichment are sown on selective, enriched and differential medium. Gram staining slide is set up and syndromic panel for meningitis/encephalitis in nested multiplex PCR is started for the rapid identification of the most common etiological agents. The volume of CFS used in each reaction is 200 µL and the run time is about 1 h.
Results: 40-year-old cancer patient, myasthenics following thymoma, immunosuppressive therapy, comes to the E.R. in 13.07.2022 with neurological symptoms such as a headache, altered mental status, lethargy. Neurological consultation is required. Blood chemistry tests are normal. The chemical-physical examination of CSF is suggestive for meningitis: opalescent appearance, hypoglycorrachia (7 mg/dl), hyperprotidorrachia (192 mg/dl) and elevated cell count (>50 cell/mmc, mainly PMNs). At Gram staining slide are visible capsulated sporulating yeasts. Syndromic panel in PCR is positive for *Cryptococcus neoformans/ gattii*. From the cultural examination on Sabouraud Dextrose Agar, incubated at 37°C for 24-48 h, the colonies are creamy, glossy, roundish, white. The microorganism, identified by automatic system as *C. neoformans*, was found to be sensitive to amphotericin B, fluconazole, itraconazole, and voriconazole, resistant to caspofungin, tested using microdilution in broth.
Discussion and Conclusions: In non-immunocompromised people, spontaneous healing occurs in about a week, while in immunocompromised patients Cryptococcal meningitis is a serious disorder with high mortality and thus best managed by an interprofessional team that includes a emergency doctor, microbiologist, disease specialist, neurologist and a pharmacist. Early diagnosis has enabled timely intervention with targeted therapy. The patient is treated in combination therapy with antifungal agents (amphotericin B + fluconazole). The patient was subsequently transferred to a centre specialised in infectious diseases.

147 - Effect of Vitamin C on dormant states of Helicobacter pylori

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Effect of Vitamin C on dormant states of *Helicobacter pylori*

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Introduction. *Helicobacter pylori* shows capability to colonize human gastric mucosa, producing biofilm and entering in dormant state that permit a long self-preservation, overcoming stressful conditions such as antibiotic administration. Recently, Vitamin C (VitC) has showed the capability to reactivate dormant bacteria by stimulation of cell respiration and, in *H. pylori*, the combination of VitC with antibiotic seems to interfere the eradication rate. The first aim of this work was to evaluate, in *H. pylori*, the progression of physiological changes from active state to Viable But Non-Cultivable (VBNC) and Persister (AP) states, establishing times and conditions to enter dormant state. Finally, Vit C ability to interfere with dormancy generation and to resuscitate dormant *H. pylori* were investigated.
Materials and Methods. The clinical MDR *H. pylori* 10A/13 coming from our lab collection was used in this study (ID Number RICH9RTLH). The antibacterial effect (MIC/MBC) of VitC was evaluated and the dormant states of *H. pylori* were induced by: nutrient starvation (for VBNC generation) incubating in: i) unenriched medium (BB without 2%FCS); (ii) sterile saline solution (SS-0.9%NaCl) and (for AP generation) antibiotic treatment at high concentration 10xMIC amoxicillin (AMX). The samples were monitored after 24-48-72h, 8 and 14 days by OD_{600}, CFUs determination, LIVE/DEAD staining under fluorescent microscopy and MTT viability test. In order to determine the VitC ability to interfere with the VBNC and AP generation, ¼ MIC of VitC was added to *H. pylori* suspension before the generation time (t= 0) or after dormant states generation and monitored after 24, 48, 72h.
Results. In *H. pylori*, the most suitable conditions to induce the VBNC state were in SS for 8 days and for the AP state at 10xMIC AMX for 48h, with a morphological transition from bacillary to viable coccoid form. VitC was able to affect the entry in VBNC state reducing the viable coccoid formation also associated to death of bacillary forms. In AP cells, VitC delayed the entry with a decrease of viable coccal cells and more bacillary and U-shaped bacteria. On induced dormant states, VitC acted in a different way: on VBNC state, it was capable to induce resuscitation (60% more than the control); whereas on AP state, VitC did not resuscitate dormant bacteria but it acted reducing the large aggregates typical of AP state.
Discussion and Conclusions. *H. pylori* shows wide dynamic physiological states which are significantly modified in presence of VitC. VitC acts reducing the entry in dormant states enhacing the resuscitation rate. The pretreatment with VitC in the *H. pylori* therapeutical schemes could favour the selection of microbial vegetative forms more susceptible to treatments.

This work was funded by Research Grant PRIN 2017 SFBFER (Prof. S. Stefani) MIUR, Italy.

148 - Mechanisms involved in the reversal of antimicrobial resistance in Acinetobacter baumannii by albumin nanoparticles

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Mechanisms involved in the reversal of antimicrobial resistance in *Acinetobacter baumannii* by albumin nanoparticles

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Introduction Infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) are a global problem in healthcare settings. The narrow range of treatments has forced the use of colistin (Col) as last-resort antibiotic, and increasing development of Col resistance has been reported. Nanotechnology is a promising strategy to counteract resistant bacterial infections. We developed a new formulation of human albumin nanoparticles for Col delivery (Col / haNPs), effective on Col-resistant (Col R) bacteria, in particular *Acinetobacter baumannii (Ab)*. The aim of this study focused on the relationships between Col / haNPs modes of action and bacterial resistance mechanisms. **Materials and methods:** Five clinical MDR-Col R strains of *Ab* sensitive to Col/haNPs were phenotipically caractherized for Col resistance mechanisms. *Ab ATCC 19606* was used as a Col-sensitive (Col S) control. The susceptibility profile for free Col and Col/haNPs was determined using the broth microdilution method according to the breakpoint defined EUCAST. LPS amount was quantifyed by sensitivity to vancomycin and pattern of growth in the presence/absence of Col by E-test. The mcr-1- mediated resistance was identified by a Col predifussion assay and EDTA incubation (CPD-E test). Phenotypic detection of efflux pump was performed using the efflux inhibitor carbonyl cyanide 3 chlorophenylhydrazone (CCCP) and the EtBr-agar cartwheel assay. For genomic characterization, Whole Genome Sequence analysis (WGS) of Col R strains was performed. To investigate the antibacterial mechanisms of Col/haNPs, we followed the interaction haNPs-*Ab* by TEM imaging and evaluated the release of reactive oxygen species (ROS) (DCFH-DA assay) and outer membrane permeabilization (NPN assay). Efflux studies were performed using EtBr as substrate. **Results:** Col/haNPs exhibited a higher antibacterial activity when compared to free Col. A significant decrease in the MIC values was observed for *Ab* Col R strains (>40 to 2.5/1.25 μg/mL Col vs. Col/haNPs). Phenotypic and genomic characterization of *Ab* Col R strains indicate that all strains were negative for the presence of *mcr-1* gene and were not LPS-deficient. WGS analysis identified in all strains genes related to drug efflux system (AcrAB, Emr, TolC) and lipid A (lpx, pmrAB,phoP,) with SNPs implicated in Col R. All strains displayed high frequency of ARGs genes. Accordingly phenotypic tests highlight a role of efflux pump in our Col R strains and indicate that haNPs restore Col sensitivity inhibiting efflux pumps. Moreover, results of TEM analysis indicate localization of the NPs on both bacterial surface but also inside cells, which further leads to cell breakage. Outer membrane permeability assay showed no permeabilization by Col/haNPs while confirmed the effect of free Col. Instead, both free Col and Col/haNPs induce an increase in ROS production. **Discussion and Conclusions:** Col/haNPs combat microbes via multiple mechanisms

that are simultaneously active. NPs can adhere to the surface of bacterial cells to produce ROS and damage the composition and structure of the bacterial cell. Moreover, efflux pumps are indeed inhibited by haNPs. Further studies will be needed to demonstrate the nanoparticle entry mechanism and the possible action of NPs on gene expression and metabolism of bacterial cells.

152 - Complete Genome Sequence of Lactobacillus crispatus Type Strain ATCC 33820

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Complete Genome Sequence of *Lactobacillus crispatus* Type Strain ATCC 33820

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Introduction: *Lactobacillus crispatus* is the most frequently isolated species among the vaginal lactobacilli of the human microbiota of healthy women; its presence is associated with reduced risk of preterm delivery, viral sexually transmitted infections, and bacterial vaginosis. To date (June 2021), only eight *L. crispatus* complete genomes are available in the NCBI database (https://www.ncbi.nlm.nih.gov/genome/browse#!/prokaryotes/1815/). Here, we contribute to the genomic characterization of this species by publicly releasing the genome of strain ATCC 33820, the type strain of Lactobacillus crispatus.
Materials and Methods: The complete genome sequence of *Lactobacillus crispatus* type strain ATCC 33820 was obtained by combining Nanopore and Illumina sequencing technologies. Nanopore and Illumina sequencing generated 136,000 long reads (630,559,194 bp; N_{50}, 8.7 kb) and 762,936 read pairs (2 × 250 bp), respectively. Nanopore reads were filtered using Filtlong v0.2.0 with the parameter-target_bases to retain a total of 230 Mbp (https://github.com/rrwick/Filtlong) (N_{50}, 19,822 bp) and assembled using Unicycler v0.4.7. The resulting circular contig was polished using Medakav 0.7.1 (https://github.com/nanoporetech/medaka) with all Nanopore reads, followed by two polishing rounds with Pilon v1.22 using the Illumina reads. Assembly quality was evaluated using Ideel (https://github.com/mw55309/ideel). Annotation was performed with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.1. Default parameters were used for all software unless otherwise specified.
Results: The genome of *L. crispatus* ATCC 33820 consists of a single circular chromosome (2,239,089 bp) with an overall GC content of 37.0%. The assembly contains 2,194 open reading frames, 78.8% with putative biological function, 64 tRNA genes, 3 rRNA operons, and 3 structural RNAs.
Discussion and Conclusions: The characterization of genomes is of fundamental importance for the taxonomy and in the context of comparative genomics. A complete genome represents a finished product in which the order and accuracy of each base has been verified, constituting an important resource for the understanding of biochemical diversity, virulence and pathogenetic and evolutionary mechanisms of microorganisms

156 - Antimicrobial and antivirulence effects of Hop extract against multi-drug resistant Staphylococci strains and Cutibacterium acnes

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Antimicrobial and antivirulence effects of Hop extract against multi-drug resistant Staphylococci strains and *Cutibacterium acnes*

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Abstract

Introduction: The increase of multi-drug resistant (MDR) and tolerant bacteria represents a global challenge that strongly underlines the need to search non-antibiotic strategies. In particular, significant is the number of MDR Gram-positive microorganisms coming from skin infections with a high percentage of methicillin-resistant *Staphylococcus aureus* (MRSA) and MDR *S. epidermidis*. These bacteria are also often found with *Cutibacterium acnes* in different skin diseases. The worrying resistance/tolerance phenomenon has raised the attention on the antimicrobial and antivirulence effects of plant extract such as Hop extract. The aim of this work was to evaluate the antimicrobial and antivirulence effects of the hydroalcoholic Hop extract of *Humulus lupulus* L. variety *cascade*, grown in Abruzzo region (Italy), against *S. aureus* and *S. epidermidis* MDR strains, and against *C. acnes*. Materials and methods: The Hop extract was phytochemical characterized by reversed phase HPLC-fluorimetric method and its biocompatibility and cytotoxicity were studied by using *Artemia salina* L. and two cell lines through MTT assay. Docking analysis was used to identify the mechanism of action. The antimicrobial effect was studied by the detection of inhibition zones and MICs against six clinical strains (four MRSA and two MDR *S. epidermidis* strains) and *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *C. acnes* ATCC 11827. The antivirulence action was determined by: membrane fluidity change by Laurdan Generalized Polarization (GPexc); anti-biofilm activity by biomass quantification and cell viability. Results: The main components of Hop extract, biocompatible and non-cytotoxic at all tested concentrations, were: gallic acid, resveratrol and rutin. The docking analysis showed high affinity of rutin against PBP2a and KAS III with Ki values in the submicromolar range. The tested Hop extract displayed good antimicrobial action with remarkable inhibition zones with all tested strains and MIC values ranging from 1 to 16 □g/ml with a not significant action in membrane fluidity. The extract also showed a remarkable anti-biofilm effect with percentages of reduction up to 99.34%±0.26 at MIC value. The Live/Dead images showed a relevant inhibition effect in the biofilm formation with a weak killing action. Discussion and Conclusions: Hop extract exhibited an interesting antibacterial and antivirulence action against the tested MDR strains suggesting it's a possible role in the prevention of infection caused by MDR Gram-positive pathogens.

159 - The administration of Enterococcus faecium SF68 counteracts compositional shifts in the gut microbiota of diet-induced obese mice

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The administration of *Enterococcus faecium* SF68 counteracts compositional shifts in the gut microbiota of diet-induced obese mice

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Introduction. Microorganisms with probiotic properties are eliciting an increasing interest as coadjuvants in the prevention and treatment of obesity through modulation of the gut microbiota. In this study, a probiotic formulation based on *Enterococcus faecium* SF68 was administered to mice fed with a high-fat diet (HFD) to evaluate its efficacy in reducing body mass gain and in modulating the intestinal bacterial composition.

Materials and methods. Both stool and ileal samples were collected from untreated and SF68-administered HFD-fed mice. Bacterial genomic DNAs were extracted from samples and absolute abundances of specific *taxa* constituting the gut microbial *consortium* evaluated through 16S rDNA-targeting Real-Time qPCRs.

Results. SF68 administration significantly reduced the HFD-induced weight gain. In these animals, the microbial gut composition shifted towards an enrichment in microbes positively correlated with mucus thickness, lower inflammation, lower glycemia levels, and SCFA production (*i.e., Bifidobacterium*, *Akkermansia*, *Faecalibacterium*), as well as a depletion in bacterial phyla having a key role in obesity (*i.e., Firmicutes*, *Proteobacteria*).

Discussion and conclusion. Our results demonstrate the efficacy of *E. faecium* SF68 in adjusting the composition of the dysbiotic microbiota of HFD-fed animals, thus ameliorating clinical conditions and exerting anti-obesity effects.

161 - Klebsiella pneumoniae ST37: a never-ending story

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Klebsiella pneumoniae ST37: a never-ending story

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Introduction: The first reports of carbapenem resistant Enterobacterales in the Policlinico Umberto I (PUI) hospital date back to 2006. In that period, few *K. pneumoniae* ertapenem-resistant and meropenem-susceptible strains were identified and assigned to sequence type (ST) 37. These produced CTX-M-15, OmpK35 was depleted due to a nonsense mutation, and a novel OmpK36 variant was identified. From 2010, KPC-producing ST512 isolates started prevailing and ST37 apparently vanished from PUI. Since 2018 the clinical use of ceftazidime-avibactam (CZA) has been implemented for the treatment of bacteria producing serine- beta-lactamases.

Yet KPC-producing, CZA resistant *K. pneumoniae* are emerging. We report about an outbreak caused by four CZA-resistant and KPC-producing ST37 *K. pneumoniae*.

Materials and methods: Seven *K. pneumoniae* ST37 isolates (three sampled in 2011 and four in 2021) were subjected to Whole Genome Sequencing (Illumina and Oxford Nanopore Technologies) and to genomic analyses. Furthermore, bla_{KPC} gene cloning in *Escherichia coli* and transformation of the bla_{KPC} carrying pKpQIL plasmid were performed to assess the role of KPC variants in the CZA-resistant phenotype.

Results: The seven ST37 strains were highly related, clustering together in a SNP-based phylogenetic tree. Contemporary and historical isolates shared the same outer antigens (03b and KL38), and only few major genomic differences were detected in the chromosome (two different prophages and the ICE*Kp* mediated acquisition of the virulence locus *ybt* in the current isolates). Contemporary ST37 isolates acquired bla_{KPC} on identical pKpQIL plasmids, distinguishable only for the bla_{KPC} gene. Specifically, 3 variants of the KPC enzyme have been identified: KPC-31 in isolates 1016 and 1021, KPC-70 in isolate 1015 and a novel KPC variant, KPC-110, in isolate 1020. The KPC variants showed a D179Y mutation in the omega loop compared to KPC-3, coupled with a T268A substitution in KPC-70 and a R43G substitution in KPC-110. In *E. coli* KPC-70 and KPC-110 displayed higher MICs for CZA than KPC-31, yet all these variants did not confer resistance to carbapenems

Discussion and Conclusions: The analysis of the evolution of CZA resistance in the once-dominant clone ST37 demonstrates that this clone is very flexible, proposing 3 different KPC variants in four isolates. Contemporary and historical ST37 isolates were highly related, hinting that ST37 survived unnoticed in our hospital for 10 years, waiting for the selection of mutant bla_{KPC} to re-emerge as a CZA-resistant *K. pneumoniae* clone. The genomic dissection of this clone highlighted its evolutionary pathways (the acquisition of the pKpQIL plasmid and of the *ybt* locus) and its development (with the KPC-70 and KPC-110 variants) to high CZA MICs.

166 - Antibacterial efficacy of glycine airflow and hyaluronic acid-vehicled antibiotic mixture on contaminated titanium surfaces of dental osseointegrated implants

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Antibacterial efficacy of glycine airflow and hyaluronic acid-vehicled antibiotic mixture on contaminated titanium surfaces of dental osseointegrated implants

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Introduction. Dental implants are exposed to numerous oral bacteria, which can colonize the titanium (Ti) surface leading to peri-implantitis:a chronic inflammatory process therefore, due to a polymicrobial infection that involves the soft and hard tissues around the osseointegrated implants. The inflammatory process leads to the formation of a peri-implant pocket with bone loss.Therefore, complications of biofilm formation are prolific in implantology, accounting for a quarter of all infections each year.The decontamination of the implant surface and the consequently resolution of the inflammation represent the main goal that has to be achieved in the treatment of peri-implantitis. Different methods have been reported to minimize or even completely remove biofilms from contaminated surfaces.Implantoplasty remains a preferred way to remove infected contaminants and the surface quality of the implant after decontamination is considered an important predictor of future outcome.Despite some positive effects, a complete resolution of the pathology is not usually achieved.The treatment of peri-implantitis is still a challenge and a final therapy remains a matter of discussion.The purpose of this *in vitro* study was to compare the effect of different decontamination protocols on the disinfection of treated acid (MAC) and etched acid (SLA) Ti disks contaminated and assess the biocompatibility of MAC/SLA disks with adhesion and grow of Adipose derived Mesenchymal Stem cells (ASCs).**Materials and Methods**.Samples were infected with a polymicrobial biofilm to simulate *in vitro* a peri-implantitis.17 MAC and 20 SLA Ti disks were randomly assigned to three different decontamination protocols: Glycine powder air-flow (GYPAP), a local delivered triple paste antibiotic composed by ciprofloxacin, metronidazole and clarithromycin (3MIX) and a combination of GYPAP + 3MIX. Biocompatibility of the Ti disks after each protocols was assessed by measurement of adhesion and grow of ASCs after 24 and 72 h.Confocal laser scanning microscope (CLSM) was used to evaluate the antibacterial effectiveness of treatment regimens.Ti disks were analyzed with Scanning electron microscopy (SEM) to visualize the presence of biofilm on the surface after 3 different decontamination treatments.**Results.** The best viability data were achieved by the 3MIX and GYPAP combination on the SLA surfaces after 72 h.CLSM analysis showed a mean ratio of dead bacteria statistically higher in 3MIX+GYPAP group compared with GYPAP and 3MIX subgroups.In SEM images 3MIX+GYPAP disinfection protocol appeared more efficient in removing bacteria.**Discussion and Conclusions.** Data showed that the combination of GYPAP and 3MIX should may be preferred to the other protocols, especially in presence of SLA titanium surface.

172 - Comparative evaluation of antimicrobial, antiamoebic, and antiviral efficacy of ophthalmic formulations

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Comparative evaluation of antimicrobial, antiamoebic, and antiviral efficacy of ophthalmic formulations

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1. Introduction. Ocular infections are becoming increasingly challenging to treat due to limited drug penetrability through corneal stroma and the appearance of resistant bacterial strains. In addition to the emerging drug resistance, antibiotics are also ineffective against parasites and viruses, making urgent a valid alternative for ophthalmic treatment. In this context, antiseptics are gaining more attention because they have a non-selective mechanism of action preventing bacterial resistance, moreover they combine parasite-killing and virucidal effects. The most used antiseptics in ophthalmology are povidone-iodine (PVP-I) and chlorhexidine (CHX), which we tested along with a natural antiseptic molecule, thymol. In the present study, we compared the *in vitro* antibacterial, antiameobic, and antiviral activities of six ophthalmic formulations containing the abovementioned antiseptics. **2. Materials and methods.** To assess their activities, Gram-positive and Gram-negative bacteria, the amoeba *Acanthamoeba castellanii*, and two respiratory viruses were enrolled. In particular, we evaluated the antibacterial activity of the ophthalmic solutions by broth microdilutions assay, disk diffusion assay and challenge test. The amoebicidal effect was assessed by determining the minimum concentration required to inhibit 50% of *A. castellanii* trophozoites replication. A plaque assay was finally used to evaluate their activity against the human adenovirus 2 (HAdV-2) and the human coronavirus OC43 (HCoV-OC43). **3. Results.** Among the tested formulations, Dropsept, consisting of Vitamin E TPGS-based (tocopheryl polyethylene glycol succinate) in combination with chlorhexidine, showed the highest range of activities, as it works efficiently against bacteria, amoeba, and viruses. On the other hand, the solution containing PVA (polyvinyl alcohol) and thymol showed an antimicrobial wide spectrum with a promising inhibitory effect on *Pseudomonas aeruginosa*. Also, thymol-based formulations were shown to be effective against *A. castellanii*. Regarding the antiviral activity, also Iodim exerted inhibition of viral replication for both respiratory viruses. **4. Discussion and conclusions**. Altogether our data suggest that, given its multiple activities, Dropsept might be a valuable alternative to treat ocular infections. Promising results were also shown by thymol, which deserves deeper investigations.

173 - Nocardia infections in a University Hospital in NorthEast Italy

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Nocardia infections in a University Hospital in NorthEast Italy

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Introduction

Nocardiosis is a rare infection that can have severe morbidity and mortality, in particular among patients with compromised immunity. Since the route of infections can vary, involving soft tissue injuries and inhalation of contaminated aerosol, primarily affected organs are the lungs, the skin, and soft tissues; From the first site Nocardia can invade the bloodstream and disseminate to any organ. In Italy, nocardiosis has not a high prevalence, but most of the reported cases have an elevated morbidity. Furthermore, like other age and immunosuppression related diseases, it is bound to increase its relevance in the near future.

In this study, we analysed cases of nocardiosis that were diagnosed at the Microbiology Unit of Azienda Ospedale Università Padova (AOPD) between 1/2017 and 5/2022.

Materials and Methods

We retrospectively analysed the cases of nocardiosis that occurred in AOPD, interrogating our data base and noting the sex, age, clinical conditions, antibiotic susceptibility of the strains. The susceptibility was determined using both microdilution in broth method and E-TEST strips.

Results and Conclusions

Table 1 Cases occurred in AOPD 2017-2022

Case	Sex	Age	Site of Infection	Nocardia spp.
1	F	87	lungs	*N. farcinica*
2	M	59	skin	*N. farcinica*
3	M	54	brain	*N. abscessus*
4	M	79	lungs	*N.spp*
5	M	70	lungs	*N.spp*
6	M	69	lungs	*N. abscessus*
7	M	80	brain	*N. farcinica*
8	F	60	lungs	*N.spp*
9	F	68	lungs	*N.spp*
10	F	80	lungs and skin	*N. farcinica*
11	M	50	lungs	*N.spp*

Nocardiosis showed to be slightly more frequent in men (M/F : 7/4), and a compromised immune state appears to be a major risk factor. This seems more related to clinical conditions and immunosuppressant drugs, while the age seems to play a minor role (median age 69 +/- 12 years). Regarding the site of the infection, 70% involved the lungs and 20% the brain. *N. farcinica* was the most common specie identified in our series, followed by *N. abscessus*. *N. farcinica* showed a general resistance to beta-lactams as opposed to *N. abscessus* strains displaying a higher sensitivity to these antibiotics. Towards Imipenem the sensibility of both species was variable, Ciprofloxacin too seemed to be not very effective. Linezolid, Cotrimoxazole, and Amikacin were the most effective molecules. MICs determined with ETEST strips had a tendency to underestimate the MIC values, in particular of beta-lactams.

In conclusion, in AOPD lungs and brain are the most common site of nocardiosis and N. *farcinica* is the most common isolate. *N. farcinica* strains showed a general resistance to beta-lactams and variable susceptibility to carbapenems, thus empirical therapy for nocardiosis should take into account Linezolid, Cotrimoxazole, and Amikacin that resulted the most effective antibiotics.

177 - Characterization of multidrug-resistant Escherichia coli strains isolated from mares with fertility problems

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Characterization of multidrug-resistant *Escherichia coli* strains isolated from mares with fertility problems

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Introduction: Bacterial infections, mainly caused by opportunistic or commensal microorganisms, are frequently associated with equine fertility problems, and *Escherichia coli* (*E. coli*) has been described as the main pathogen. Mares are prone to developing chronic infections due to bacteria capability to produce biofilm, which confers the ability to microorganisms to evade the immune system and to resist to antimicrobial therapy. Furthermore, the pathogens need essential pathogenicity traits, including adhesion and invasion to host cells, motility mediated by flagella and toxins, to manifest the disease. This study aimed to define the phenotypic and genotypic characterization of *E. coli* strains isolated from mares suffering from fertility problems.

Materials and Methods: *E. coli* strains were isolated from routine bacteriological examinations of uterine swabs collected from mares diagnosed with reproductive disorders. A total of 24 *E. coli* strains were collected in 2018. The isolates were characterized for antibiotic resistant profile using the simple disk diffusion technique as recommended by the Clinical and Laboratory Standards Institute guidelines for veterinary isolates (CLSI 2015). Biofilm formation ability was established by crystal violet staining. The presence of some virulence factor genes was assessed according to multiplex PCR assay.

Results: The antimicrobial resistance profiles showed high resistance to penicillin (95.8%), ampicillin (95.5%), imipenem (91.7%), amoxycillin/clavulanic acid and tetracycline (41.7%). Whereas the most efficient antimicrobial was amikacin (100% of cultures), followed by enrofloxacin (95.8%), ofloxacin (91.7%) ceftiofur (86.9%) and gentamicin (75.0%). In addition, 54% (13/24) of the isolated *E. coli* resulted to be multidrug-resistant strains. Only two out of the total of 24 *E. coli* isolates showed haemolysis on blood agar. The majority of *E. coli* strains 71% (17/24), were strong or moderate biofilm producers; among them 65% (11/17) were MDR strains. Furthermore, PCR analysis showed the presence of bacterial virulence factor genes such as *kpsMTII*.

Discussion and Conclusions: This study characterized *E. coli* strains from mares diagnosed with reproductive disorders. Some of them showed a multidrug-resistant profiles, carried virulence genes and were strong and moderate biofilm producers. The high resistance revealed by almost all strains to imipenem underline the need to expand this

investigation to a larger number of isolates and to deepen the characteristics of virulence of these strains. A better understanding of bacteria can guide the adoption of more effective management practices and treatment strategies.

181 - Omic-insights of Ceftazidime-Avibactam resistant Klebsiella pneumoniae clinical isolates

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Omic-insights of Ceftazidime-Avibactam resistant *Klebsiella pneumonia*e clinical isolates

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Introduction

In the last decade, multiple-drug-resistant *Klebsiella pneumoniae* strains emerged as an important cause of healthcare-associated infections worldwide. In particular, *K.pneumoniae* carbapenem resistant (KPC) strains complicate the treatment of infections. In 2018, ceftazidime/avibactam (CZA) was approved by the Italian authorities to cure infections sustained by KPC. Unfortunately, CZA exposure rapidly increased the emergency of CZA-resistant strains. Herein, we characterize resistome and virulome of the KPC-producing CZA-resistant strains selected under CZA treatment in three hospitals of Southern Italy, by molecular approach.

Materials and Methods

Sixteen isolates were collected from three hospital located in Catania and Catanzaro between May 2020 to October 2021. Isolates were collected from 13 patients who developed CAZ resistance during treatment. Antimicrobial susceptibility was determined by broth microdiluition method against a panel of antibiotics. The samples included in the study were analyzed for Resistome, Virulome and to determine Sequence Type (ST) by Whole Genome Sequencing (WGS).

Results

Molecular analysis showed circulation of three major clones ST101, ST307 and ST512. In ten of sixteen strains we found bla_{KPC-3} gene; in the remaining samples we detected four different KPC variants (28, 31, 34 and 50). A plethora of Beta-lactams genes ($bla_{SHV28-45-55-100-106-187-205-212}$, $bla_{OXA1-9-48}$-$bla_{TEM-181}$ and $bla_{CTX-M-15}$) were found variably associated with the different genetic profile while bla_{OXA-9} was found in ST307 and 512. With regards to membrane permeability, we investigated *omp*K35 and *omp*K36 that present frameshift mutations in 15 out of 16 strains; *omp*K37, all strains harbored a non-functional protein. Our isolates possessed wild-type PBP3.

Discussion and Conclusion

In this study, we reported the occurrence of CZA-resistance in a group of KPC isolates, serially collected during the pandemic period in two regions of Southern Italy. All isolates belonged to different genotypes and all of them were further analyzed to characterize their virulome and resistome. In all CZA-R isolates, MER/VAP and cefiderocol remained in the range of susceptibility, demonstrating to maintain reliable *in vitro* activity against these difficult to treat isolates. The enhanced use of CZA, would cause an emergence of concerning resistance. The results of this study provide further evidence for the plasticity and the evolutionary potential of clone of *K.pneumoniae*. In conclusion our data would prompt a further characterization of carbapenem resistance and the intrinsic bacterial factors that facilitate the rapid emergence of resistance, together with improving the timely detection of these KPC variants, contributing differently to the resistance phenotype.

183 - Oral hygiene, dietary habits, dental caries experience, and quantitative determination of microbial species with high cariogenic potential in children with autism spectrum disorder

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Oral hygiene, dietary habits, dental caries experience, and quantitative determination of microbial species with high cariogenic potential in children with autism spectrum disorder

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Introduction: Communication deficits, poor social interactions, repetitive unusual behaviors and high sensitivity to external stimuli render extremely challenging the dental care of children with Autism Spectrum Disorder (ASD), demanding for early identification of subjects at high risk of caries development in order to implement timely preventive measures. The aim of the present study was to evaluate the oral hygiene and dietary habits, the caries status, the occurrence in saliva and the salivary count of microbial species with cariogenic potential in a group of Italian children with ASD attending the Pediatric Dentistry Outpatient Clinic, reserved for patients with ASD, "Sharing to cure" path, of Pisa's University Hospital.

Materials and Methods: 52 patients with diagnosis of ASD and of 29 age-matched neurotypical subjects, mainly represented by patient's siblings were recruited. Questionnaires were administered to mothers to inquire about the eating and oral hygiene habits of their children. Each subject underwent a careful intra-oral examination by a dentist specifically trained to deal with ASD subjects in order to take record of the oral health status. A commercial saliva-check buffer kit was used for determination of saliva pH and buffering capacity. *Mitis salivarius* bacitracin agar and *Candida* ident. agar medium supplemented with gentamicin were used for the selective recovery of *Streptococcus mutans* and *Candida spp* respectively. The study is under approval by the local Ethics Committee and was in accordance with the Declaration of Helsinki.

Results: Among the variables assessed, a statistical significant difference was recorded between ASD and control group in the use of aids other than toothbrush (e.g. floss, brush, and mouthwash), frequency of intake of sugary drinks and probiotics, salivary pH, oral hygiene index and fraction of decayed teeth on the total number of teeth. In a sub-group of 22 ASD and 13 controls tested so far, the occurrence of *Streptococcus mutans* and *Candida* spp was higher in ASD children than healthy controls, although the difference did not reach statistical significance. Overall, the salivary counts of *S. mutans* were low with only few subjects exhibiting levels of the bacterium higher than 10^5 colony-forming unit (CFU)/ml, considered predictive of high caries-risk.

Discussion and conclusions: The results obtained support the view that ASD children are at higher risk of caries development than healthy controls, although dedicated pathways aimed at improving oral hygiene and cooperation during dental treatments may contribute to keep low the oral colonization by cariogenic species.

186 - Ceftobiprole non-susceptibility in Enterococcus faecalis is influenced by PBP4 alterations

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Ceftobiprole non-susceptibility in *Enterococcus faecalis* is influenced by PBP4 alterations

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Introduction
We previously demonstrated that elevated ceftobiprole (BPR) MICs were attributable to increased *pbp*4 transcription, associated with a single upstream adenine deletion (*del*A). PBP4 substitutions in the catalytic-site *motifs* alter the protein folding and stability and may account for the (already low) PBP4 affinity to BPR, and MIC increase. Here we aim to correlate how these aminoacidic substitutions, especially those falling near the catalytic site, interfere with the formation of the BPR/PBP4 complex.
Materials and Methods
Two already characterized *Enterococcus faecalis* clinical isolates were selected for their β-lactam resistance profile, *pbp4* gene sequence and key mutations influencing the BPR antibacterial activity. *pbp4* gene expression was performed by RT qPCR and the relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. BPR/PBP4 binding affinity was analyzed in a competition assay with increasing concentrations of BPR (sub-MIC; 1X, 2X and 4X MIC). PBPs were labelled using the fluorescent reporter molecule BOCILLIN™ FL.
Results
The comprehensive analysis of *pbp4* gene sequence, expression levels of transcription, and competition assays revealed that the coexistence of the *delA* upstream the promoter region and aminoacidic substitutions near the catalytic-site significatively affect the formation of the BPR/PBP4 complex. The Penicillin-Resistant-Ampicillin-Susceptible/BPR-Non-Susceptible (PRAS/BPR-NS) strain exhibited the adenine deletion (*del A*) in the promoter region and 4 different aminoacidic substitutions, among which 2 felt next to the PBP4 catalytic-site *motifs*. *delA* leads to a 3 $\log_{10}$ increase such that the *pbp4* expression levels of transcription were upregulated, consistently to its higher BPR MICs (MIC 16 mg/L). Aminoacidic substitutions next to the PBP4 catalytic-site *motifs* clearly interferes with the BPR/PBP4 affinity, leading to a saturation of PBP4 at lower MIC concentrations. The Penicillin-Susceptible-Ampicillin-Susceptible/BPR Susceptible (PSAS/BPRS) did not show *delA* and showed only one aminoacidic substitution, far from the catalytic site. This strain maintains lower *pbp*4 expression levels ($\leq 10^2$ fold-change increase) and full BPR susceptibility (0.25 mg/L). Its PBP profile is not altered, showing an increasing saturation of PBP4, proportionally dependent to BPR concentrations.
Discussion and Conclusions
Our data suggest that diverse factors converge in determining reduced susceptibility to ceftobiprole, in specific *E. faecalis* strains: increased expression of low-affinity PBP4, as a result of *del*A in the promotor region, and over-reduced BPR/PBP4 binding, due to aminoacidic substitutions next to the catalytic-site *motifs*.

Keywords: Enterococcus faecalis; PBP4 substitutions; competition assay; RT-qPCR.

188 - Blastocystis sp. prevalence and subtypes in autochthonous and immigrant patients in a Southern Italy hospital

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Blastocystis sp. prevalence and subtypes in autochthonous and immigrant patients in a Southern Italy hospital

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Introduction: *Blastocystis* (Stramenopiles) is a common enteric protist transmitted by fecal-oral direct contact or waterborne transmission with a worldwide distribution. Although several publications suggest an association with gastrointestinal symptoms (i.e. diarrhea, abdominal pain, nausea, vomiting, fatigue, flatulence, irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), the clinical significance of *Blastocystis* and of its subtypes (STs) still remains a controversial issue. Since the pathogenicity has not been fully elucidated, testing for *Blastocystis* is not routinely pursued by most laboratories and clinicians. Thus, the *Blastocystis* prevalence is underestimated. The aim of the study is to explore the prevalence of *Blastocystis* and characterize the genetic diversity in autochthonous and immigrant patients, evaluating: *i*) the influence of the geographical origin, *ii*) the dynamic of different STs and *iii*) the association with other parasite coinfections.
Material and methods: From February 2022, an individual stool sample of autochthonous and immigrant patients with gastrointestinal symptoms admitted to the Microbiology and Virology Unit was screened for the presence of *Blastocystis* sp. by using Allplex™ GI-Parasite Assay Real Time. The samples found positive were subjected to traditional PCRs targeting a partial fragment of the SSU rRNA gene and then subjected to the BygDye Terminator sequencing in order to identify and characterize *Blastocystis* subtypes.
Results: Until now, out of 60 screened samples, n. 13 (21.6%) have been found positive to *Blastocystis* sp. The prevalence was higher in male (n. 9, 69.2%) compared to female (n. 4, 30.7%) and in Italian (n. 10, 77%) compared to African (n. 3, 23%) patients. Moreover, out of 13, 1 (7.7%) has found also positive to *Dientamoeba fragilis*. Two *Blastocystis* subtypes were identified with the following distribution: ST3 in 11 samples and ST1 in 2 samples.
Discussion and conclusions: *Blastocystis* was detected in both the Italian and African group patients. Our data confirmed previous studies performed in Italy, in which ST3 proved to be the most prevalent subtype, but we highlight also the detection of ST1 subtype, which was probably underestimated in former analyses. We found an association between *Blastocystis* and *D. fragilis* that might indicate a potential cooperative interaction between these two enteric protozoa. In order to explore the potential clinical relevance of *Blastocystis* and its subtypes, the association with other protozoa and also the possible changes of gut microbiota associated to *Blastocystis* infection further prospective studies with a higher number of samples will be performed.

190 - In vivo antibacterial activity of disposable gauzes for periocular antisepsis

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***In vivo* antibacterial activity of disposable gauzes for periocular antisepsis**

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Introduction. Disposable gauzes for periocular antisepsis can be useful for removing debris of eyelid desquamation or secretions due to inflammatory events or allergies or as adjuvant in the treatment of eye pathologies such as conjunctivitis, blepharitis, and blepharoconjunctivitis. Herein we evaluated the effect of gauzes containing natural extracts from *Citrus limon* (Biosecur), *Aloe vera*, and *Ruscus aculeatus* in reducing the bacterial load of the conjunctiva and eyelids of patients undergoing intravitreal injections for maculopathies.

Materials and Methods. Swabs from conjunctiva and eyelids of both right and left eyes of patients were collected before the treatment to assess differences in the microbial colonization. The eye that needed to receive the intravitreal injection was treated 4 times a day for 4 days with the gauzes and sampling was repeated for both eyes at the end of the treatment. The eye not receiving the treatment was used as control. Bacterial counts were performed by plating. Bacterial genomic DNAs were extracted from the samples and Real-Time qPCRs performed to analyze the composition of the microbial community in terms of total bacterial load and major taxa.

Results. Before treatment, no differences between right and left eyes were evidenced in any patient and both samples from eyelids and conjunctiva displayed *Staphylococcus* spp. as predominant bacteria. A significant reduction in bacterial counts was evidenced after the use of the antiseptic gauzes both on eyelids and conjunctival samples, with *Staphylococcus* spp. almost undetectable after treatment. Molecular analyses are still in progress.

Discussion and Conclusions. In the present study, the application of the antiseptic gauzes was shown to be effective in reducing the bacterial load of the eyelids and the conjunctiva. While effects on the eyelid may be due to direct contact of the active principles contained in the gauzes with bacteria as well as to mechanical removal, we hypothesized that the reduction of bacterial load on conjunctiva may be a result of the spread of the active principles from the eyelid and the periocular area to the conjunctiva itself, thus conferring to the antiseptic device a wider and more effective antimicrobial effect. Although the *in vivo* antibacterial effect of the tested gauzes and the usefulness of this kind of treatment for periocular antisepsis have been clearly demonstrated, the results of Real-Time qPCRs will be useful to better comprehend the effect of the treatment on different bacterial populations.

192 - Cyano-pyrimidines and enamines as promising scaffolds for antibacterial activity

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Cyano-pyrimidines and enamines as promising scaffolds for antibacterial activity

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Introduction

The combination of virtual screening and chemoinformatic with biology has finally led to practical applications in workflows for prioritizing narrow subsets from large chemical spaces. Pyrimidine- imidazole- and enamine-based derivatives are privileged scaffolds that attracted considerable attention in designing of molecules, with DNA replication or bacterial cell division inhibitory properties. A series of cyano-pyrimidines, cyano-imidazoles and enamines was previously selected by virtual and experimental screening (Research Centre for Natural Sciences, Budapest) and Enamine Ltd. covalent fragment libraries, resulted in several hits with potential antibacterial activity. In this study, we show a preliminary evaluation of these compounds as suitable starting points for developing new antibiotics.

Materials and Methods

27 compounds (N. 10 cyano-pyrimidine, N. 5 cyano-imidazole and N. 12 enamine derivatives) selected from a covalent docking-based virtual screening workflow, using different *state of art* softwares, were explored for their antibacterial properties against eight microbial reference strains from ATCC (American Type Culture Collection). Three independent experiments were performed to calculate Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Results

n. 5 cyano-pyrimidine and n. 7 enamine derivatives showed promising antibacterial activity against some standard bacteria. No activity was achieved for cyano-imidazoles. We found that overall, almost all candidates (11/12) exhibited potential antibacterial activity against *A. baumannii* species, inhibiting the growth at 64 μg mL^{-1} concentration, and being bactericidal at the same concentration or one concentration above the MIC (125 μg mL^{-1}). Three cyano-pyrimidines showed specific activity against *E. faecalis* (16, 32 and 64 μg mL^{-1}). One compound (4-chloropyrimidine-2-carbonitrile) showed a broad-spectrum activity also against *P. aeruginosa* and *C. albicans* strains (MIC 64 μg mL^{-1}). Enamine derivatives were specifically active against Gram-negative bacteria, killing *A. baumannii* at 64 μg mL^{-1} and inhibiting also *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *C. albicans* (5/7). No antimicrobial activity was found at relevant concentrations against *S. aureus* and *B. subtilis* species (MIC ≥256 μg mL^{-1}).

Conclusions

Given the rise in antibiotic resistance, our promising results, particularly against *A. baumannii*, open the way to further development of cyano-pyrimidine and enamine derivatives as optimal candidates for the treatment of Multidrug Resistant (MDR) clinical isolates.

Keywords: Virtual screening; Covalent Docking; antibacterial activity.

196 - Lytic activity of four bacteriophages against planktonic and biofilm Staphylococcus aureus clinical isolates

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Lytic activity of four bacteriophages against planktonic and biofilm *Staphylococcus aureus* clinical isolates

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Introduction: Phage therapy represents a promising approach to cure difficult-to-treat infections. In 2019, *Staphylococcus aureus* became the second most commonly found pathogen causing lethal antibiotic-resistant infections worldwide. This is primarily due to its capacity to either develop multidrug-resistance or to form biofilm, in which bacterial cells are more tolerant to antibiotics in comparison to their planktonic counterparts. The aim of the study was to evaluate the host range and the lytic activity of 4 established staphylococcal bacteriophages against a panel of *S. aureus* strains.
Material and methods: 4 phages, ISP, Sb-1 (Eliava Phage Therapy Center), Romulus and Remus (KU Leuven), all belonging to the *Twortvirinae* subfamily, were employed in this study. A 20 *S. aureus* strains dataset, including 2 strains from type culture collections and 18 clinical isolates from 4 countries (Belgium, Germany, Italy, and Switzerland), was used. The phage host range was evaluated by performing a spot assay of each phage against all strains at 3 titers (10^8-10^6-10^4 PFU/ml) and assessing their susceptibility by the presence of lysis plaques. A 24h growth kinetic was performed to determine the activity of each single phage and a combination (cocktail) of all of them (10^8-10^6 PFU/ml) against planktonic cells by measuring culture optical density (OD). Finally, antibiofilm activity of ISP was assessed against 2 strains, treating their biofilms (preformed on porous glass beads) with 3 titers (10^8-10^7-10^6 PFU/ml) for 24h.
Results: The spot assay revealed that all bacterial strains were sensitive at least to 2 phages and 15 out of 20 strains were susceptible to the highest tested titer of all 4 phages. ISP and Sb-1 had the broadest host range, being active against all 20 strains. Growth kinetic assays showed that the OD value of 9 strains incubated with at least one phage did not increase within 24h, suggesting that phages were able to kill planktonic bacteria. Moreover, the phage cocktail, when provided at both titers, had a bactericidal effect against all strains tested. ISP showed an antibiofilm activity against both tested strains, resulting in complete biofilm eradication for one strain and a 3-log reduction for the other, relative to the untreated control.
Discussion and Conclusions: Our results provide evidence that the tested phages have rather wide host ranges and exhibit lytic activity against the *S. aureus* strains tested. Moreover, the ability of phage cocktail to inhibit cell growth in all cases suggests a synergistic effect of the phages, which may differ in their mechanism of interaction with the target bacteria, increasing the probability of infection. Further experiments to evaluate the antibiofilm activity of staphylococcal bacteriophages are ongoing.

201 - Klebsiella pneumoniae strains resistant to ceftazidime/avibactam and meropenem/vaborbactam: molecular characterization.

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Klebsiella pneumoniae strains resistant to ceftazidime/avibactam and meropenem/vaborbactam: molecular characterization.

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INTRODUCTION

In the last decades, multidrug-resistant (MDR) Gram-negative bacteria recorded high distribution rates among clinical isolates. Beta-lactamases and carbapenemases production slightly decreased the effectiveness of beta-lactams and carbapenems. Specifically, *Klebsiella pneumoniae* carbapenemases (KPC) became a global public health concern. Several studies showed excellent activity of ceftazidime/avibactam and meropenem/vaborbactam against KPC-producers with low resistance percentages. The isolation of two MDR hypervirulent *K. pneumoniae* resistant to ceftazidime/avibactam (CZA) and meropenem/vaborbactam (MER/VAB) attracted our attention. Here we report their first molecular characterization.

MATERIALS AND METHODS

Two isolates of *K. pneumoniae* were collected from patients recovered respectively in the Internal Medicine Unit in Civico Hospital (Palermo) and in the Hematology ward in University Hospital Policlinico (Catania). Clinical specimens were respectively a blood sample and a rectal swab. *In vitro* susceptibility testing and NGS sequencing were performed. Illumina DNA Prep was used for sequencing analysis, and the pooled libraries were sequenced on Illumina MiSeq. Data were analyzed using QIAGEN CLC Genomics Workbench software, Microbial Module.

RESULTS

Original *in vitro* susceptibility tests performed in the hospital clinical laboratories revealed multi-drug resistance extended to novel beta-lactam/beta-lactamase inhibitor combinations. The strains were resistant to 3d generation cephalosporins, meropenem, CZA and MER/VAB.
Molecular analysis revealed the following similar characteristics: isolates belong to ST101 clone, featured with capsular locus KL-17 and O-antigen locus O1/O2v1. We found carbapenem genes bla_{KPC3} and bla_{OXA-48} and beta-lactams gene

bla$_{SHV1.}$ Moreover, membrane permeability was investigated with attention to *omp*K35, *omp*K36, *omp*K37 and frameshift mutations were detected. Otherwise, the absence of mutation was confirmed on PBP3 sequences.

DISCUSSION AND CONCLUSION

The clinical impact of new molecules' resistance leads to significant difficulties in the therapeutical management of infections. Although low prevalence rates have been currently recorded, our data implement evidence already reported in the literature. We detected resistance mechanisms related to the presence of carbapenems genes *bla*$_{kpc3}$ and *bla*$_{OXA48}$ and beta-lactams gene *bla*$_{SHV1.}$ Furthermore, frameshift mutations on *omp*K35, *omp*K36, and *omp*K37 genes were found. This study highlights how the presence of multiple resistance mechanisms on bacterial strains should be always investigated. Whole genome sequencing could represent an additional step analysis to perform anytime an unusual susceptibility pattern is clinically revealed.

208 - Pleurotus eryngii var. thapsiae a new potential culinary-medicinal mushroom from Sicily with antibiofilm potentiality.

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Pleurotus eryngii var. _thapsiae a_ new potential culinary-medicinal mushroom from Sicily with antibiofilm potentiality.

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Introduction: Mushroom extracts are a rich source of natural compounds with antimicrobial properties. In recent years, there has been an increased interest in natural antimicrobials, especially those obtained from medicinal and culinary mushrooms. The genus *Pleurotus* (Fr.) P. Kumm. includes economically important mushrooms, cultivated around the world, widely used in human nutrition and recognized as medicinal mushrooms. Within this genus, the *P. eryngii* species-complex is undoubtedly worth mentioning. Among the members of the *P. eryngii* species-complex, our attention focused on *P. eryngii* (DC.) Quél.var. *thapsiae* Venturella, Zervakis & Saitta, this mushroom has been reported so far only in Sicily (Italy), where it has a rather limited geographical distribution, at altitude ranging from 0 to 1500 m. The aim of this study was to investigate the potential of extracts from *P. eryngii* var. *thapsiae*, against ATCC Gram-positive and Gram-negative strains.
Materials and Methods: The cultivation was carried out on two different substrates, one based on wheat straw and another consisting of a mix of wheat straw and *Aegilops geniculata* Roth., a wheat weed. *P. eryngii* var. *thapsiae* exhibited good production performance, with yields above 15% on both substrates. Besides, the collected basidiomes were subjected to drying, then reduced to powder and a chemical analysis was then performed on this powder. One share of the mushroom powder was used to obtain aqueous extracts using two different techniques, traditional and ultrasound-assisted extraction, in order to evaluate and compare their activity against ATCC Gram-positive (*S.aureus*, *E faecalis)*, and Gram-negative (*K pneumoniae*, *E. coli* and *P.aeruginosa)* strains.
Crystal violet assay (CVA) was executed to quantify biofilm formation by all ATCC isolates
Results: The best activity was obtained against *S. aureus*, with a reduction of about 60% of biofilm biomass, by means of both ultrasonic extracts.
Conclusions: Extract of mushroom or their purified antimicrobial constituents are alternative biocides that have recently gained attention as possible cleansing agents, because the chances of resistance development in bacterial cells could be minimal. This study, therefore, highlights the potential application of this mushroom for large-scale cultivation, aimed at the marketing of a local, quality product with high nutritional and organoleptic properties.

210 - Profile of antibacterial activity of Moringa oleifera Lam. single phenols against the phytopathogen Xanthomonas campestris pv. campestris

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Profile of antibacterial activity of *Moringa oleifera* Lam. single phenols against the phytopathogen *Xanthomonas campestris pv. campestris*

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Introduction: *Xanthomonas campestris pv. campestris* (XCC) is the phytopathogenic agent causing black rot in crucifers; once reached the target site in the plant, it damages the plant cells causing maceration of tissues and obstruction of the xylematic vessels. The research of alternative to pesticides/antibiotics has led to the study of plant extracts with antibacterial properties, and with this optic, the effects of extracts of *Moringa oleifera* Lam. (MO) against XCC have been studied. Knowing which phenolic compounds are mostly present in our MO extracts, i.e., chlorogenic acid, ellagic acid, rutin and quercetin, in the project we investigated the effects of the single phenols constituting the phytocomplex on bacterial cells.

Materials and methods: The potential antimicrobial effects on biofilm and membrane permeability on XCC of several phytochemical phenolic compounds have been evaluated: chlorogenic acid (CA), ellagic acid (EA), rutin (RU) and quercetin (QU) have been studied.

Result and Conclusions: The four different phenolics compounds have both bacteriostatic and bactericidal effects at the concentrations of 10 µg/ml for RU, 50 µg/ml for QU and CA and 100 µg/ml for EA. All of the compounds detected in MOs show effects on inhibition in biofilm formation processes and lead to a significant alteration of the bacterial membrane. It is assumed that the effect is carried out at several levels: these phenolic compounds are in fact capable of altering the permeability of the membrane leading to a halt in the ATP-synthesis, resulting in slowing down of all ATP-dependent functions. The modification of membrane integrity and permeability results in a considerable energy dissipation as it involves the dissipation of the action potential and the alteration of the electrochemical gradient, necessary conditions for the synthesis of ATP. This alters various ATP-dependent mechanisms, such as biofilm formation: XCC, subjected to these energy shortages, retains a capacity to form biofilms reduced by 87% (RU), 71% (CA), 60% (QU) and 54% (EA).

212 - Blastocystis sp. subtypes detected from 2016 to 2018 and correlation to intestinal disease.

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***Blastocystis sp*. subtypes detected from 2016 to 2018 and correlation to intestinal disease.**

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Introduction. *Blastocystis sp*. is often reported as the most commonly detected intestinal protozoa in humans and animals, despite pathogenicity continues to be debated and investigated.
Among the 17 known subtypes (STs), 9 were found in humans, with ST1-ST4 the most prevalent and ST5-ST9 isolated sporadically and likely related to zoonotic transmission. The aim of this study was to carry out the analysis of subtypes of *Blastocystis sp*. detected in human faecal samples.
Materials and Methods. DNA extracted from 104 *Blastocystis sp*. positive faecal samples (about the half of *Blastocystis sp*. positive specimens collected in a 2-year period) was submitted to subtyping by sequencing the 600 bp fragment of the small subunit rDNA gene. The samples belonged to 102 patients suspected of having an intestinal parasitosis (75 males, 27 females; 94 adults, 8 children; 41 born in Italy, 61 born in developing countries, 35 of them attending to an immigrant health service, 5 with a documented recent arrival in Italy) and submitted to conventional methods for the diagnosis of intestinal parasitoses. Some of those revealed also coinfections by other parasites (45 cases).
Results. The subtypes found in 103 of the 104 sequences obtained belonged to ST1-ST4. Among the analysed cases, ST3 was the most frequent (41.7%), followed by ST1 (30.2%), ST4 (17.7%), and ST2 (15.6%). In 1 case a ST14 was revealed in an Italian 33-year-old woman. ST3 was the prevalent strain among males, foreigners, and adults; ST4 was the prevalent ones among females and Italians, and ST2 in children. Each subtype seems not to be related to the presence of a co-infecting parasites; however, subtype 4 was mainly detected in absence of parasites other than *Blastocystis* . Among the people attending the immigrant health service and/or recently arrived in Italy, 62.8% was infected by ST3 (55% of all the ST3 infected patients). There seems to be no correlation between ST and clinical signs and symptoms among the cases analysed in this study.
Discussion and Conclusions. The frequency rate of the STs found in this study is similar to that previously reported in Italy and, except in 1 case where a zoonotic strain (St14) was revealed, only the STs most common in humans were encountered (ST1-ST4). However, among the different subpopulations (sex, origin, age) of this study the STs ratio was different. This study responds to the need to increase the data and consequently the knowledge about the subtypes circulating in Italy, where still few studies in the field have been undertaken.

215 - Characterization of fosfomycin-resistance mechanisms in clinical isolates of methicillin-resistant Staphylococcus aureus

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Abstract

Characterization of fosfomycin-resistance mechanisms in clinical isolates of methicillin-resistant *Staphylococcus aureus*

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Introduction. Methicillin-resistant *Staphylococcus aureus* (MRSA) was among the first problematic multi-resistant pathogens emerged in the clinical setting, causing infections associated with high morbidity and mortality rates. Intravenous fosfomycin, due to its notable anti-staphylococcal activity and favorable safety and pharmacokinetic profiles, is an interesting agent for combination antimicrobial chemotherapy against some MRSA infections. In this work we investigated fosfomycin susceptibility and resistance mechanisms in a multicenter collection of MRSA from Italy. **Materials and Methods.** A total of 64 non-replicate clinical isolates of MRSA *Staphylococcus aureus* collected from Hospital-acquired pneumonia cases from different Italian hospitals in 2016 were studied. Fosfomycin susceptibility testing was performed by reference agar dilution according to the ISO-20776-1:2019 guidelines and interpreted according to EUCAST clinical breakpoints (12.0). Bioinformatic analysis was carried out on genome assemblies of the 64 isolates that had previously been generated by WGS analysis. Gene expression experiments were carried out by RT-Real Time PCR. Selection of fosfomycin-resistant mutants was carried out using an overnight culture plated on fosfomycin selective agar plates. **Results.** Overall, 22% (14/64) of the MRSA isolates resulted resistant to fosfomycin. Bioinformatics analysis on resident *uhpT*, *glpT*, *murA*, *tet38*, and *fosB* genes, detected 6 distinct alterations putatively associated with resistance in the *uhpT* and *tet38* genes. Of these, three alterations introduced a premature stop codon in the *uhpT* gene, one was characterized by a deletion of part of *uhpT* gene and two caused aminoacidic substitutions in the Tet38 efflux pump. Overexpression of *tet38* was detected in two isolates. The resident *fosB* gene was detected only among isolates belonging to CC8, CC5 and ST30 (in total 29 of the 64 MRSA isolates), of which only six were resistant to fosfomycin. Interestingly, in two of them a *fosB* overexpression was mediated by an IS*1182* insertion upstream of the *fosB* gene, which provided an additional promoter. Mutant-selection experiments were able to obtain fosfomycin-resistant mutants by the same mechanisms from two susceptible strains. **Discussion and Conclusion.** In conclusion, in this work a high fosfomycin resistance-rate in MRSA from Italy was detected. Multiple fosfomycin-resistance mechanisms were identified, including *uhpT* gene alterations, *tet38* gene alterations and overexpression, and upregulation of the *fosB* gene mediated by an IS*1182* transposition upstream of the gene.

219 - Cupferron, a new promising metal chelator against Candida albicans

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Cupferron, a new promising metal chelator against *Candida albicans*

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Introduction: *Candida albicans* (*C. albicans*) represents a life-threatening opportunistic fungal pathogen with a high mortality and morbidity rate, particularly among immunocompromised patients. The high multidrug resistance combined with the limited drugs available calls for the urgent need for new therapeutic strategies. As part of the ongoing search for new antifungal drugs, we focused on metal chelators, whose activity has already been documented. In this context, the antifungal activity of N-nitroso-N-phenylhydroxylamine (Cupferron) and its effect on fungal virulence were assessed against *C. albicans* strains. The obtained data suggested that Cupferron could represent a promising drug for the treatment of *C. albicans* infections.

Materials and Methods: The cytotoxicity of Cupferron was evaluated via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) on VERO cells and hemolysis assays on human erythrocytes (400 – 1.56 µg/mL). The antifungal potential was assessed via disk diffusion test, broth microdilution method, time-killing assay and live-dead evaluation. Cupferron effect on phospholipase and catalase activity, as well as on yeast-to-hyphal transition was estimated.

Results: Cupferron exhibited cytotoxicity and hemolysis rates below 18% at concentrations below 200 µg/mL. The minimum inhibitory concentration (MIC) of the compound was 50 µg/mL, exerting fungistatic action**.** MIC and sub-MIC concentrations of Cupferron significantly reduced the activity of phospholipase and catalase and hyphae formation.

Discussion and Conclusions: Cupferron impairs fungal growth and some virulence factors involved in the invasiveness of *C. albicans*. Given the low rates of cytotoxicity at active concentrations and the growing interest in the search for new antifungal drugs, Cupferron could represent a promising candidate for *C. albicans* infections.

220 - Fourier Transform Infrared (FT-IR) Spectroscopy as new approach to analyze the clonal relationship of Citrobacter koseri and real time outbreak tracing

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Fourier Transform Infrared (FT-IR) Spectroscopy as new approach to analyze the clonal relationship of *Citrobacter koseri* and real time outbreak tracing.

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Introduction. *Citrobacter* are opportunistic pathogens, we can identify different groups of patients that suffered for *Citrobacter* associated infection and almost everyone present immunocompromised situation. *C. koseri* is an opportunistic pathogen that shows the highest tropism for Central Nervous System among *Citrobacter* species. Nowadays the rapid identification of possible hospital or community outbreak is important to take preventive measure in order to contain the spread of specific strain. The study aims of evaluate the Fourier Transform Infra-Red (FT-IR) approach for the detection of clonal relationship of *C. koseri* strains.

Material and methods. 107 *C. koseri* strains were included in the study and they were isolated from different specimen. The 107 *C. koseri* were divided into 2 groups: 56 strains were clonally related with different DNA typing technique, 51 were not genotypically related with the first group.

To investigate the clonal relationship among the isolated strains was performed three different genetic approaches: Pulsed Field Gel Electrophoresis (PFGE), core genome Multi Locus Sequence Typing (cgMLST) and PCR that target a region that belong only to a particular clonal strain. To keep preanalytical variable as standardized as possible, spectra acquisition with FT-IR (IR Biotyper, Bruker) was performed from strains grown on Tryptic Soy Agar medium at 37°C for 24h±1h. Data analysis of acquired spectra were performed with different statistical tools that are already included in the software: Euclidian single linkage (dendrogram), Principal Component Analysis (2D or 3D scatter plot and deviation plot) and Linear Discriminant Analysis (LDA). To evaluate the concordance among the FT-IR and the genetic typing methods (PFGE and cgMLST) we calculate the Adjusted Random coefficient (AR), Adjusted Wallace coefficient (AW) with corresponding Confidence Interval (CI) and Simpson's Index (SI).

Results

We start from known genetic data, in order to set up an epidemiological analysis using a new proteomic approach named FT-IR spectroscopy

Among the 56 clonal strains, 52 were grouped in a cluster and four of them form a new cluster. This event may occur due to biochemical modification in the microorganism, which were not detected by genetic typing, but further analysis may clarify this theory. During the analysis of non-clonal strains, we also discovered the presence of another cluster composed by 12 isolates. The clonality of the new cluster was confirmed with PFGE, except for an isolate. We found out also two cluster composed each by two isolates that were confirmed related by PFGE analysis.

Conclusions. FT-IR may represent a valid alternative to classical genetic typing method, especially for the saving of time, which allow performing a real-time epidemiological analysis. This study implement the few data available nowadays about the concordance between DNA based typing method and FT-IR biotyping. This rapid typing analysis produce a real time epidemiological surveillance, which may be an important factor in prevention of large spread of strains that may be highly pathogenic or that may carry Multi Drug Resistance

221 - Prevalence and antimicrobial profile of Pseudomonas aeruginosa in clinical isolates collected at Ruggi d'Aragona Hospital in 2015-2019 period

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Prevalence and antimicrobial profile of *Pseudomonas aeruginosa* in clinical isolates collected at Ruggi d'Aragona Hospital in 2015-2019 period.

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Introduction: *Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative, ubiquitous environmental and opportunistic pathogenic bacteria. Able to infect several human districts, especially, the respiratory and the urinary tract, and skin, in immunocompromised patients it can provoke persistent infections. Being a biofilms producer bacterium, *P. aeruginosa* is involved in clinical field and nosocomial infections. Its aggressiveness is also due to its inherent antibiotic resistance and to a large repertoire of virulence factors. The present retrospective study focusses the *P. aeruginosa* presence among samples collected at Ruggi d'Aragona hospital in Salerno, its resistance profile with the relative variations during the five years analyzed.
Material and Methods: samples collected from patients by 0-98 years in the 2015-2019, resulted positive for PA growth, were analyzed. Bacterial identification and antibiotic susceptibility tests were carried out by VITEK® 2. Database and statistical analysis were performed by Excel and R software, respectively.
Results: In the 2015-2019 time frame, 1621 isolates of *P. aeruginosa* resulted from respiratory samples (33.6%)**,** wound swabs (21.3%,) urine culture (16.9%), cultural swab (12.4%), blood and liquor culture (7.5%), catheters (3.9%), vaginal swabs (2.7%), and others (1.5%). Male resulted more susceptible, with an incidence of 58.7%, in respect to the female (41.3%). The isolated strains resulted low resistant versus amikacin (17.4%), gentamicin (25.4%), and cefepime (28.2%), moderately resistant versus ceftazidime (34.3%), imipenem (35%), and piperacillin/tazobactam (37.6%), highly resistant versus ciprofloxacin (42.5%) and levofloxacin (49.7%).
Discussion and Conclusion: Albeit picks recorded during the five years, a decreasing trend was observed for ceftazidime, imipenem, gentamicin, and amikacin. Several new alternative strategies for ESKAPE treatment are underway. Monitor the sentinel germs in own reality, their frequency, distribution and antibiotic resistance trend variations over time is useful to optimize their antibiotic treatment, avoiding the antibiotic resistance phenomena spread

222 - Biofilm formation, genetic signatures, and antimicrobial resistance profile of Neisseria gonorrhoeae strains

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Biofilm formation, genetic signatures, and antimicrobial resistance profile of *Neisseria gonorrhoeae* strains

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Introduction *Neisseria gonorrhoeae* is able to produce biofilms thus might be considered as a factor leading both to persistent of gonorrhea infections as well as influence the antimicrobial resistance profile. This study aims to evaluate the relationship between biofilm-forming capacity, the presence of some specific genetic signature by whole-genome sequencing (WGS), and the antimicrobials resistance profiles in *N. gonorrhoeae*. **Materials and Methods** A sample of 22 gonococci was analysed together with 5 World Health Organization (WHO) reference strains. The Biofilm Ring Test (BRT) method was used to quantify the biofilm production of *N. gonorrhoeae in vitro*, the attachment and initial biofilm formation in the presence of DNase, and to assess the kinetic of early biofilm formation. The phenotypic characterization

of gonococci was assessed by gradient diffusion methods (E-Test and MIC Test Strip) to define the antimicrobial susceptibility profiles. The genome sequencing was performed by Illumina NextSeq 500 platform, and the genomes were analyzed on https://pubmlst.org/organisms/neisseria-spp/. **Results** Strong biofilm producers (SBPs) were 71.4%, while 28.6% were classified as weak biofilm producers (WBPs). The cut-off for SBP classification was defined as three standard deviations above the mean BRT values obtained for the *N. gonorrhoeae* ATCC 49226 reference strain. In addition, DNase I treatment caused a significant ($P < 0.001$) reduction compared with proteinase K in the initial microbial attachment and biofilm formation. This suggests that biofilm formation in *N. gonorrhoeae* depends more on extracellular DNA (eDNA) than on protein production. Among SBP, four gonococci showed a decreased susceptibility to cefixime and resistance to ciprofloxacin. The majority of them belong to genogorup (G)1407 and to the *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) ST90 and Multi Locus Sequence Type (MLST) ST1901. The core genome MLST (cgMLST) scheme, which utilizes a set of 1649 loci, highlighted a high genetic diversity of gonococci. By genomic analysis of the target genes involved in biofilm production, we observed that the allele type 12 of the *nagZ* gene, which is involved in gonococcal biofilm disassembly, was predominant among the WBP producers. **Discussion and Conclusions** The results represent a first step beyond the state-of-art of antimicrobial resistance providing a novelty in the evaluation of *N. gonorrhoeae* resistant isolates.

223 - In vivo evolution of ceftazidime-avibactam and meropenem-vaborbactam resistance in a KPC-producing Klebsiella pneumoniae from meningoencephalitis complicated with brain abscess

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In vivo evolution of ceftazidime-avibactam and meropenem-vaborbactam resistance in a KPC-producing _Klebsiella pneumoniae_ from meningoencephalitis complicated with brain abscess

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Introduction The worldwide spread of carbapenemase-producing *Klebsiella pneumoniae* (Kp), steered by several high-risk clones, has been recognized as a worrisome public health challenge. Although the beta-lactam/beta-lactamase inhibitor combinations as ceftazidime-avibactam (CZA) and meropenem-vaborbactam (MVB) were recently introduced as novel therapeutic options against Class A beta-lactamases (e.g., KPC-type), resistance to CZA and/or MVB cases have been already reported, mostly associated with enzyme mutations (only for CZA resistance), or with porin alterations and increased bla_{KPC} expression (cross-resistance). This work reports on the characterization of a CZA-MVB resistant KPC-producing *K. pneumoniae*, selected *in vivo* during CZA therapy. **Materials and Methods** Kp KP16340 and KP16600 were sequentially isolated from rectal swabs, before and after therapy with CZA, respectively, in a patient with a KPC-producing Kp (KP16537) meningoencephalitis with brain abscess. Species identification and antimicrobial susceptibility testing were performed using MALDI-TOF MS and reference broth microdilution, respectively. Results were interpreted according to the EUCAST clinical breakpoints v.12. Meropenem (MEM) hydrolyzing specific activity was determined for KP16340, KP16357, KP16600 and Kp FIPP-1 as comparator strain. The bla_{KPC} copy number was evaluated by a relative qPCR quantification. Whole-Genome Sequencing (WGS) was performed on KP-16340 and KP16600 to verify the presence of mutations in KPC and porins and clonal relatedness, using tools of Center for Genomic Epidemiology (www.genomicepidemiology.org). Gene transfer experiments were carried out with *Escherichia coli* DH10B on Mueller-Hinton agar supplemented with ceftazidime 4 mg/L. **Results** KP16340, KP16357 and KP16600 showed CZA MICs of 4, 8 and 32 mg/L, respectively. KP16600 also presented a MVB MIC of 32 mg/L. Moreover, KP16600 showed an increased MEM hydrolysing activity (2.25-fold *vs* KP16340), due to an increased number of copies of the bla_{KPC} gene (7.7-fold *vs* KP16340). WGA analysis revealed that both KP16340 and KP16600 belonged to ST2502, a single locus variant of the high-risk clone ST101 and were almost identical in the core genome (1 SNP), with the same porin status and KPC allelic variant. The bla_{KPC}-harbouring plasmids of KP16600 were successfully transferred to *E. coli* DH10B, which, after the transfer, showed an increased MIC to CAZ/AVI (from ≤1/4 to 2/4). **Discussion and Conclusions** The increased expression of bla_{KPC-3} in KP16600, putatively due to a rearrangement of the bla_{KPC} harbouring plasmid, with the addition of a truncated OmpK35 and a GD (134-135) duplication in OmpK36, could be associated with the rapid *in vivo* development of resistance to both CZA and MVB.

228 - Niclosamide as a Repurposing Drug against Corynebacterium striatum Multidrug-Resistant Infections

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Niclosamide as a Repurposing Drug against *Corynebacterium striatum* Multidrug-Resistant Infections

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Introduction: *Corynebacterium striatum* (*C. striatum*) is an emerging multidrug-resistant (MDR) pathogen associated with nosocomial infections. Due to the depletion of effective antibiotics, drug-repurposing as an alternative approach to discovering new potential antibiotics is attracting considerable interest. Since these drugs are approved by the Food and Drug Administration (FDA), information on their pharmacological characteristics (chemical stability, toxicity, dosage, action kinetics, etc.) is readily available. This reduces the time and economic costs required to evaluate new therapeutic applications. In this scenario, we screened the antimicrobial activity of the anthelmintic drugs doramectin, moxidectin, selamectin and niclosamide against 20 *C. striatum* MDR clinical isolates. Among these, niclosamide was the best performing drug against *C. striatum*.
Material and Methods: The antibacterial efficacy was determined via disc diffusion, broth microdilution, time-killing assays and Scanning Electron Microscopy. The biofilm biomass eradicating action was investigated through crystal violet (CV), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and confocal laser scanning microscopy (CLSM). Niclosamide cytotoxicity was evaluated by MTT assay on immortalized human keratinocyte cells (HaCaT). The intracellular action of niclosamide was evaluated by gentamicin protection assay, in HaCaT cells.

Results: Among the anthelminthic drugs, doramectin and moxidectin did not exhibit effective antibacterial activity in the concentration range tested, while selamectin showed a MIC_{90} value of 6.25 μg/mL. Niclosamide represented the pharmacological agent with the best performing antibacterial activity, recording a MIC_{90} value of 0.39 μg/mL. Moreover, the drug-induced a growth inhibitory area of 22 mm in agar plates and exhibited bactericidal action after 20 hours of treatment. Niclosamide affected the biofilm viability in a dose-dependent manner and degraded biomass by 55 and 49% at 0.39 μg/mL and 0.19 μg/mL. CLSM images confirmed the biofilm biomass degradation, showing a drastic reduction in cell viability. The MTT assay after 20 h of treatment on HaCaT cells recorded a 50% cytotoxic concentration (CC_{50}) at 2.56 μg/mL. The dose of 1.56 μg/mL was sufficient to inhibit the replication of *C. striatum* into HaCat cells by

approximately 400 times, resulting in an intracellular load of 1.5×10^4 CFU / mL relative to 7×10^6 CFU / mL in untreated cells.

Discussion and Conclusions: Our findings demonstrate that niclosamide represents a safe therapeutic agent with strong antibacterial and antibiofilm activity. This study could promote the drug-repurposing of the anthelmintic FDA-approved niclosamide as a therapeutic agent to counteract the *C. striatum* MDR infections.

229 - Giving drugs a second chance: identification of drugs active against Pseudomonas aeruginosa from cystic fibrosis patients using a drug repurposing strategy

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Giving drugs a second chance: identification of drugs active against *Pseudomonas aeruginosa* from cystic fibrosis patients using a drug repurposing strategy

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Introduction. *Pseudomonas aeruginosa* causes chronic airway infections in cystic fibrosis (CF) patients. The multidrug resistance this pathogen shows does the search for novel antibiotics urgent. In the present study, a drug repurposing approach was used to find drugs active against *P. aeruginosa* under experimental conditions relevant to CF. **Materials and Methods.** A total of 3386 approved and clinical drugs (Drug Repurposing Compound Library; HY-L035; MedChemExpress, Sollentuna, Sweden) were screened, each at 0.1 mM, against *P. aeruginosa* RP73 under "CF-like" conditions - i.e., artificial sputum medium, pH 6.8, and 5% CO_2. After 24 h-incubation at 37°C, the antibacterial activity was assessed by spectrophotometric reading (OD_{620}) and tetrazolium-based assay (OD_{492}; CellTiter AQueous One Solution, Promega Italia, Milan, Italy). A potential "antibacterial hit" was identified for OD_{620} or OD_{492} reduction of ≥ 90%. The antibacterial potential of the hits was then furtherly explored against 10 *P. aeruginosa* CF strains, selected for their virulence and antibiotic-resistance traits: MIC and MBC values were measured by broth microdilution assay, whereas time-kill assay was performed against RP73 strain. The potential for cytotoxicity was assessed towards IB3-1 CF bronchial epithelial cells by CellTiter AQueous One Solution (Promega). **Results**. The initial screening towards RP73 strain identified a total of 14 (14 out of 3386, 0.4%) hits, namely drugs that have been developed for therapeutic indications other than antibacterial. Hits were classified according to their therapeutic indications (cancer, n=12; anti-infection - i.e., antiparasitic, antifungal or antiviral - n=2) and clinical information (launched, n=9; phase 3, n=5). Using a set of *P. aeruginosa* CF strains, we found that antibacterial activity was highly preserved by auranofin (MIC_{50}: 0.025 mM; MIC_{90}: 0.1 mM), followed by 5-fluorouracil (MIC_{50}: 0.025 mM; MIC_{90}: 0.2 mM), and tirapazamine (MIC_{50}: 0.05 mM; MIC_{90}: 0.2 mM). MBC_{50} values were less changeable, ranging from 0.2 to >0.2 mM, while MBC_{90} was always higher than 0.2 mM. Time-kill results showed bacteriostatic activity of most hits, except for ebselen that at 4xMIC caused 100% killing already after 2 h-exposure. Cytotoxicity tests revealed the best profile for tavaborole, L-SelenoMethionine, and 5-fluorouracil being not toxic until 8 uM. Generally, hits were not toxic at the antibacterial concentrations, except for auranofin (already toxic at 0.0126 uM).

Discussion and Conclusions. Our results defined some drugs that have the potential to be repurposed as antibacterial agents toward *P. aeruginosa* and, therefore, may represent progenitor scaffolds for new classes of anti-*P. aeruginosa* agents. *In vitro* and *in vivo* efficacy/toxicity studies are ongoing for further screening.

230 - T2Bacteria® Panel sensitivity in bone marrow transplanted neutropenic febrile patients

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T2Bacteria® Panel sensitivity in bone marrow transplanted neutropenic febrile patients

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Despite a general drop in mortality rate, sepsis is still one of the major causes of death urging for new tools that could help shortening the diagnostic turnaround time. Blood culture is still the gold standard, but many diagnostic algorithms have included molecular techniques from positive blood cultures, thus reducing average time to identify relevant pathogens and to detect genes of resistance. Other molecular diagnostics were developed to be used directly on blood samples, such as the T2Dx system.
In this study, we evaluated the T2Bacteria® panel in our diagnostic routine of neutropenic bone marrow transplanted patients.
We collected samples from patients in bone marrow transplant unit and hematology day hospital in IRCCS San Raffaele hospital. We included samples associated with first fever for each septic event. Blood samples were processed from 9.00 to 19.00 and results were referred to clinicians. No antimicrobial therapy was changed or modified according to T2Dx results. Positive blood cultures were processed during the same time shift.
Seventy-seven samples were collected, but seven were excluded due to technical issues. Comparing the results of T2Dx system with blood culture results, most were matched negative tests (49). Nine positive T2Bacteria®panels included matched positive blood culture (5) and positive tests with no matched blood culture (4). We found twelve negative tests associated with positive blood cultures with nine out of twelve species included in the panel. According to our results overall sensitivity was 50%, considering ESKAPE group only, lowering to 42% considering other bacteria. Considering off-hours (19.00 - 9.00) we esteemed an average time of blood culture positivity of 15 hours with longer times needed to process blood cultures if positivity occurred during nightshifts.
Despite other studies showed high sensitivity and considerable reduction in starting appropriate antimicrobial therapy according to T2Bacteria®results, no studies were available on bone marrow transplanted patients yet. We have not definitive explanations on the factors contributing to the low sensitivity observed, but they may include kinetics of Gram-negative bacteria during the septic event in these patients or possible drug interactions. Further studies are certainly needed. Moreover, our study highlights the importance of a 24/7 microbiology lab is crucial to fully benefit the impact of new molecular techniques. Many positive tests were in fact referred to clinicians the morning after when the matched blood culture was positive and processable for further testing

231 - Preliminary data of the prevalence of Capnocytophaga spp in kennel dogs and feline colonies of North-East Italy: emergent zoonotic bacteria?

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TITOLO: **Preliminary data of the prevalence of *Capnocytophaga spp* in kennel dogs and feline colonies of North-East Italy: emergent zoonotic bacteria?**

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1. Introduction:*Capnocytophaga spp*.(*C.spp*) is recently isolated a commensal microorganism of human and animals, some species are common in human (*C.ochracea*,*C.sputigena*,*C.gingivalis*), other in animals (*C.cynodegmi*,*C.canimorsus*, *C.canis*).This microorganism could cause infection in humans, especially in subjects with precarious health condition. Infection occurs via cat or dog bite or after a close contact with the animal's saliva. According to the literature, infection in humans could have serious complication (e.g.sepsis,endocarditis,death) which are mainly caused by *C.canimorsus (C.ca)*, while other species could cause minor infection like *C.cynodegmi (C.cy)*. As Capnocytophaga is considered as an emergent zoonosis is necessary to develop a method to specifically isolate and identify it. In this work are reported preliminary data of a study conducted in kennels dogs and feline colonies of North-East Italy, evaluating the prevalence of this microorganism in dogs and cats and to identify the present species by both molecular and culture methods.
2. Materials and methods:Oral swabs obtained from dogs and cats were analysed in IZSVe laboratory:Swabs was subjected to bacteriological examination, suspected *C.spp* colonies were identified by biochemical methods and species identification was performed by MALDI-TOF mass-spectrometry (MTMS). Moreover, the presence of Capnocytophaga was investigated by Real Time PCR using the method developed by Dam et al.(2009) based on two different species-specific PCRs for *C.ca* and *C.cy*.
3. Results:In 2021 was sampled 346 animals (44%dogs, 56%cats). By bacterial culture *C.spp* was isolated in 13% of dogs and 6% of cats. Among the positive samples, 43% are *C.cy* and remaining results to be "not identifiable". Real Time PCRs reported an overall positivity of 66% for *C.spp*: 27% result to be positive for *C.cy*, 25% as *C.ca* and remaining shown a double positivity for both species.
4. Discussion and conclusion:This work reports preliminary data of a research conducted on the prevalence of *C.spp*. in kennels dogs and feline colony in North-East area. Identification and isolation was performed with both microbiological and molecular methods. In Oral swabs by culture method *C.spp* was isolated in 13% of cases whereas by Real Time PCRs 66% of cases results as positive. Species identification by MTMS was not very efficient since only a 43% of isolates was identified as *C.cy*.Also, Real time PCR in a lot of cases shows a positivity to both C.cy and C.ca These preliminary data highlight that *C.spp* is present in oral flora of dogs and cats, hence the improvement of both bacterial culture and molecular methods to isolate/detect this bacterium is crucial in a One-Health vision.
The Italian Ministry of Health [IZSVE 12/19 RC] supported this work.

234 - Antimicrobial applications of bimetallic nanoparticles by heterogenous green synthesis

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Antimicrobial applications of bimetallic nanoparticles by heterogenous green synthesis

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Introduction: The increase of high resistant bacterial strains to antibacterial drugs seriously impacts public health. In recent years, bimetallic nanoparticles, formed through the integration of two different metals, such as silver, gold, and platinum, have shown improved antibacterial efficacy compared to monometallic ones due to the synergistic effects, the wide range of physicochemical properties, and the different mechanism of action. They have shown numerous applications in different fields from the medical to the physical one. To satisfy the increasing demand for commercial NPs, a new eco-friendly "green" method of synthesis has been evolved and it avoids environmental build-up. The aim of this study was focused on the synthesis of bimetallic silver and platinum nanoparticles (AgPtNPs) by a biological source, i.e. *Ocimum basilicum*, and the evaluation of their antimicrobial potential. **Materials and Methods:** *Ocimum basilicum* leaf extract was prepared following the protocol of Saba Pirtarighat et al. In brief, dried samples were ground to a fine powder, double-distilled water was added and the mixture was boiled for 5 min. The aqueous extracts were filtered through Whatman No.1 filter paper after cooling. To synthesize AgPtNPs in the presence of plant extract, *O. basilicum* was mixed with 1 mM $AgNO_3$ and 1 mM K_2PtCl_4 solution and stirred at 85º C for 60 minutes. The reaction was monitored with UV-Vis spectroscopy. The obtained NPs were centrifuged and washed several times with H_2O to remove any untreated salts and extracts. The pellet was then oven-dried at 40 º C to obtain the NPs powder. FTIR spectra of extract-reduced AgPtNPs were obtained using a FTIR fitted with an ATR detector in the range of 4000-500 cm-1. The NPs morphology and particle size were determined by using transmission electron microscopy (TEM) at 200kV voltage, meanwhile NPs stability was checked by using the Dynamic light scattering (DLS) technique. The antimicrobial activity of AgPt was tested by using the serial microdilution method against Gram-positive and Gram-negative bacterial pathogens. **Results:** The reduction of noble metal can be confirmed by observing the color change in the reaction. Initially, the color of *O.basilicum* was yellow, although after the addition of $AgNO_3$ and K_2PtCl_4 under constant shaking at 80º C the color change of reaction was observed turning from dark yellow to an orangish green color indicating the formation of AgPtNPs. After 1 hr of reaction time, UV-Vis spectra of extract of *O.basillicum* capped AgPtNPs showed the peak around 320-450nm. Further, TEM analysis confirmed AgPtNPs size ranged from 20 to 80nm. The NPs were stable with different and formatted spherical shape nanoclusters. The minimum inhibitory concentration (MIC) was analyzed against Gram-positive and Gram-negative bacteria, namely *Escherichia coli*, *Klebsiella pneumonia Enterococcus faecalis*, and *Staphylococcus aureus*, by testing different NPs concentrations from 0.75 to 100 µg/ml. MIC values were around 3.15 µg/ml for all bacteria. **Discussion and Conclusions:** The study has highlighted the potential of bimetallic nanoparticles synthesized by *O. basilicum* extract. Therefore, the combinational activity of AgPtNP can be used for the treatment of multidrug-resistant bacteria as a novel antimicrobial strategy.

235 - Repurposing Selamectin as antimicrobial drug against Hospital-Acquired Staphylococcus aureus infections

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Repurposing Selamectin as antimicrobial drug against Hospital-Acquired *Staphylococcus aureus* infections

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Introduction: *Staphylococcus aureus* (*S. aureus*) represents a clinically relevant pathogen in both the nosocomial and community settings. The emergence of multidrug-resistant strains requires the urgent discovery of new antibacterial drugs. Drug reuse reduces development costs and drug approval time. In this context, an antibacterial screening of anthelmintic avermectins doramectin, moxidectin and selamectin against *S. aureus* was performed.

Material and methods: The antibacterial screening of anthelmintic drugs was performed via broth microdilution method against *S. aureus* ATCC 6538 (50 - 0.4 µg/mL). Among the drugs tested, selamectin had a strong antibacterial action. Therefore, its antibacterial activity was thorough on clinical isolates via time-killing assays and Scanning Electron Microscopy. The potential target of Sekamectin was detected through PatchDock and FireDock software. The synergistic effect with Ampicillin was assessed via checkerboard test. The intracellular action of Selamectin was evaluated by gentamicin protection assay in HaCaT cells. The biofilm biomass eradicating activity was assessed via crystal violet (CV) and confocal laser scanning microscopy (CLSM).

Results: Selamectin represents a high-performance drug. Its minimum inhibitory concentration (MIC) was 6.25 µg/mL against all *S. aureus* strains, except for the macrolide resistant isolate (MIC 12.5 µg/mL), exhibiting bactericidal action. This result defined its involvement in the protein synthesis process, confirmed *in silico* through interaction studies with 23S rRNA. As a result, selamectin drug exposure caused cell wall alterations, probably due to changes of the surface protein structures. A synergistic effect was observed between the drug and selamectin, dictated by an FIC value of 0.5 against methicillin-resistant *S. aureus*. We assessed the role of selamectin in the *S. aureus* invasion of human keratinocytes via gentamicin protection assays. Drug administration at 1× MIC reduced the intracellular bacterial load by 81.3%. The biofilm degradation was investigated via crystal violet (CV) and confocal laser scanning microscopy (CLSM). Selamectin degraded the biomass biofilm in a dose-dependent manner with minimal biofilm eradication concentrations inducing 50% ($MBEC_{50}$) eradication 10.33 µg/mL. The cytotoxic tests proved that selamectin exhibited no relevant hemolytic and cytotoxic activity at concentrations below 12.5 µg/mL.

Discussion and Conclusions: These data, together with the already available *in vivo* toxicity studies, strongly state that selamectin represents the most promising macrocyclic lactone for the treatment of *S. aureus* infections.

237 - In vitro antibacterial and anti-inflammatory activity of Arctostaphylos uva-ursi leaf extract against Propionibacterium acnes

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In vitro antibacterial and anti-inflammatory activity of *Arctostaphylos uva-ursi* leaf extract against *Propionibacterium acnes*

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Introduction: *Propionibacterium acnes* (*P. acnes*) is the main causative agent of acne vulgaris. Although acne vulgaris is not a life-threatening disease, it has a great social and psychological impact on the patient's life. Today therapeutic options for this disease include the use of antibiotics with some disadvantages as dryness, redness, skin irritation and hyperpigmentation and the improper use of antibiotics for a long time leads to the development of multi-drug resistance. Contextually, the use of natural substrates as a source of bioactive compounds is arousing considerable interest nowadays. The advantages associated with the use of plant extracts concern minimal side effects in the patient, use of renewable compounds and a high degree of safety with low production costs compared to synthetic drugs. For this reason, the study aims to evaluate the antimicrobial activity of a natural product, *Arctostaphylos uva-ursi* leaf extract, against *P. acnes*.

Materials and Methods: *Arctostaphylos uva-ursi* leaf extract antimicrobial activity was evaluated via disk diffusion, microdilution broth, time-killing and live/dead assays. The influence of natural compound on biofilm matrix inhibition and degradation was investigated by the crystal violet (CV) method. The 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT) test determined the viability of immortalized human keratinocytes (HaCaT) after exposure to *Arctostaphylos uva-ursi* leaf extract, for 24 and 48 hours. Levels of interleukin (IL)-1β, IL-6, IL-8, and tumour necrosis factor (TNF)-α were quantified after heat-killed *P. acnes* HaCaT cells infection and *Arctostaphylos uva-ursi* leaf extract treatment.

Results: The minimum inhibitory concentration (MIC)-value of *Arctostaphylos uva-ursi* leaf extract against *P. acnes* was 0.6 mg/mL, showing bacteriostatic action and an inhibition zone on agar-plates of 24 ± 1.2 mm. Furthermore, the compound influenced the biofilm formation phases, recording a percentage of inhibition that exceeded 50 and 40% at 0.6 and 0.3 mg/mL, respectively. *Arctostaphylos uva-ursi* leaf extract disrupted biofilm biomass of 57 and 45% at the same concentrations mentioned above. Active *Arctostaphylos uva-ursi* leaf extract doses did not affect the viability of HaCaT cells. On the other hand, at 1.25 and 0.6 mg/mL, complete inhibition of the secretion of pro-inflammatory cytokines was recorded.

Discussion and conclusions: Taken together, these findings indicate that *Arctostaphylos uva-ursi* leaf extract could represent a natural product to counter the virulence of *P. acnes*, representing a new alternative therapeutic option for the treatment of acne vulgaris.

239 - Rapid identification of clinically relevant Candida spp. by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

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Rapid identification of clinically relevant *Candida* spp. by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

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1. Introduction. Currently Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-ToF MS) and molecular techniques are used in the routine diagnosis of clinically relevant bacteria and yeast. Attenuated Total Reflectance (ATR) Fourier Transform InfraRed (FT-IR) spectroscopy is a spectrum-based technique that quantifies the absorption of infrared light by molecules present in the microbial cell. The IR spectrum provides a specific fingerprint that reflects the cell composition of nucleic acids, proteins, lipids, and carbohydrates. The aim of the present study was to evaluate the performance of the ATR-FTIR spectroscopic technique (I-dOne 2.0 Alifax) compared with the MALDI-TOF MS (Bruker Daltonics) in identifying *Candida* spp. isolated at the Micology Unit of Pisa University Hospital.
2. Materials and Methods. A yeast colony isolated from Sabouraud agar was deposited onto the ATR crystal sampling surface of the ATR-FTIR spectrometer for identification. Spectral acquisition time was approximately 60 s (30 s for the background and 30 s for the sample) and the automated spectral processing and yeast identification required additional 30 s. Yeast identification was performed in triplicate. Each Infrared Spectrum was compared with spectra stored in the software database.
3. Results. Identification performed by I-dOne software of 133 yeast clinical isolates yielded 125 (93,9 %) concordant identification results with MALDI-TOF MS. Two isolates (2.2%) were identified as *Candida* spp. (1 *C. albicans*, 1 *C. parapsilosis*) and six isolates (4.5%) were misidentified at the species level (*2 C. albicans* as *C. psilosis complex, 2 C. glabrata* as *C. krusei*, *2 C. tropicalis* as *C. albicans*).

Discussion and Conclusions. A reliable database for the identification of clinical yeast species has been built. Additional strains belonging to species rarely found in clinical samples and not easily available from laboratory collections should be included to expand the database. The database has several valuable potentials that should be further studied in the next future.

242 - Molecular characterization of linezolid resistance determinants in Staphylococcus epidermidis from invasive infections

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Molecular characterization of linezolid resistance determinants in *Staphylococcus epidermidis* from invasive infections

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Introduction. *Staphylococcus epidermidis* is a common skin and mucosal membranes commensal, able to induce opportunistic infections mainly associated to medical devices. The recent spread of isolates resistant to several classes of antimicrobial compounds, including last resort antibiotics such as linezolid is of concern. Since now, linezolid resistance in *S. epidermidis* has been associated to 23S rRNA, L3, L4 and L22 mutations or the acquisition of transferrable resistance determinants (mainly the methyltransferase Cfr). Therefore, the aim of this work was to characterize the mechanisms of linezolid-resistance of *S. epidermidis* isolated from invasive infections. **Materials and Methods.** Samples collection included *S. epidermidis* strains isolated from clinical specimens collected from June 2014 to July 2021 in the AOU-Careggi Hospital showing a linezolid-resistant phenotype tested with automated devices. Linezolid resistance was confirmed by reference broth microdilution assays and interpreted according to EUCAST clinical breakpoints. The most common Gram-positive acquired oxazolidinone resistance genes were searched by Real-time PCR (*optrA*, *poxtA*, *cfr* and *cfr*(B)). A subgroup of isolates was subjected to Whole Genome Sequencing (WGS) using an Illumina platform and raw reads were assembled using SPAdes-software. Bioinformatics analysis for the detection of resistance mechanisms was performed by BLAST, MLST, RESfinder and LREfinder tools. **Results.** A total of 77 *S. epidermidis* isolates were included in the study. All the strains showed high level of resistance to linezolid (MIC >8mg/L), but only 12 of them tested positive for the presence of *cfr* gene. WGS was performed on 15 isolates. All strains belonged to ST-2 and the presence of the *cfr* gene was confirmed in 4 isolates, while the other strains did not show any acquired resistance gene. G2603T mutation of the 23S rRNA gene were detected in 13/15 isolates, while only few strains showed additional mutations in the L3 and L4 proteins (8 and one, respectively). No mutations in the L22 ribosomal protein were detected. **Discussion and Conclusions.** These data highlight a high prevalence of previously reported chromosomal mutations and few Cfr-producing isolates (15.6%) leading to linezolid resistance. Novel chromosomal mutations were also identified in most of the isolates underlying the impact of the selective pressure induced by linezolid usage. Epidemiological surveys should be performed to limit the spread of acquired resistance mechanisms and monitor the raise of novel mutations. WGS analysis on the remaining isolates will be necessary in order to investigate the molecular origin of linezolid resistance and their clonality.

245 - Prevalence and Antimicrobial Resistance Profile of Enterococcus Species: A Retrospective Cohort Study in Italy

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Prevalence and Antimicrobial Resistance Profile of Enterococcus Species: A Retrospective Cohort Study in Italy

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Background

Antimicrobial resistance represents one of the main threats to healthy ecosystems. In recent years, among the multidrug-resistant microorganisms responsible for nosocomial infections, the *Enterococcus* species have received much attention. Indeed, *Enterococcus* have peculiar skills in their ability to acquire resistance genes and to cause severe diseases, such as endocarditis. This study showed the prevalence and antimicrobial resistance rate of *Enterococcus* spp. isolated from clinical samples, from January 2015 to December 2019 at the University Hospital “San Giovanni di Dio e Ruggi d’Aragona” in Salerno, Italy.

Methods

Bacterial identification and antibiotic susceptibility were performed with VITEK 2. Statistical analysis was done using SPSS (IBM Corp, Armonk, NY). A chi-square test was used to compare the differences antibiotic sensitivities over the range of years considered in the study.

Results

A total of 3236 isolates of *Enterococcus faecalis* (82.2%) and *Enterococcus faecium* (17.8%) were collected from urine cultures, blood cultures, catheters, respiratory tract, and other samples. *E. faecium* showed a high resistance rate against ampicillin (84.5%), ampicillin/sulbactam (82.7%), and imipenem (86.7%), while *E. faecalis* showed the highest resistance rate against gentamicin and streptomycin high level, but both were highly sensitive to such antibiotics as tigecycline and vancomycin.

Conclusions

Studies of surveillance are an important tool to detect changes in the resistance profiles of the main pathogens. These antimicrobial susceptibility patterns are necessary to improve the empirical treatment guideline of infections.

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246 - Bacterial Coinfection and Antibiotic Resistance Profiles among COVID-19 Patients

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Bacterial Coinfection and Antibiotic Resistance Profiles among COVID-19 Patients

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INTRODUCTION: Many studies showed that microbial co-infections in COVID-19 patients can aggravate the symptomatology and clinical course of the disease. The purpose of this retrospective study is to evaluate the prevalence of bacterial coinfections and associated antibiotic resistance profiles among COVID-19 patients admitted to the University Hospital “San Giovanni di Dio e Ruggi d’Aragona”, Salerno, Italy between April 2020 and June 2021.

MATERIALS AND METHODS: Age, gender, bacterial identities, and antibiotic sensitivity profiles were collected retrospectively for 358 hospitalized COVID-19 patients. Identification of microorganisms and antibiotic sensitivity tests were performed with Vitek 2. The IBM Statistical Package for Social Sciences Version 22.00 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS: The co-infection rate was higher in male patients (64.5%) aged 60 to 80 years (62.5%). Among them, microbial co-infection events are distributed as follows: bacteremia (33.9%), pneumonia (28.5%), bacteriuria (28.2%), skin and soft tissue infections (5.4%) and others (4%). Out of 806 isolated species, 431 (53.5%) were Gram-positive bacteria, 359 (44.5%) were Gram-negative bacteria and 16 (2.0%) were mycetes. The microorganisms most frequently isolated from the lower respiratory tract and blood cultures are *Staphylococcus aureus* and Staphylococci coagulase negative (CoNS), while *Escherichia coli* and *Enterococcus faecalis*, have been more isolated from urine cultures. Antimicrobial sensitivity tests showed high rates of resistance to erythromycin (88.3%) and oxacillin (70.8%) by *Staphylococcus aureus isolates* and excellent sensitivity to linezolid (100%) and vancomycin (97.1%). In addition,

Escherichia coli isolates showed highest sensitivity to Fosfomycin (100%), and a high rate of resistance to piperacillin (81.3%) and cefuroxime (73.9%).

CONCLUSIONS: The data showed increased rates of antibiotic resistance compared to the values reported in studies prior to the pandemic period; in particular, an increase of prevalence of methicillin-resistant *Staphylococcus aureus* and extended-spectrum ß-lactamase producing *Enterobacteriaceae* were reported. In conclusion, these data could be used to improve empirical antimicrobial therapy and co-infection management strategies in COVID-19 patients, and finally, prevent the spread of multi-resistant bacteria.

248 - Carbapenem-resistant and extended-spectrum β-lactamase producing Klebsiella pneumoniae: Prevalence and Antimicrobial Resistance trends in Strains Isolated from Hospital in Italy.

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Carbapenem-resistant and extended-spectrum β-lactamase producing *Klebsiella pneumoniae*: Prevalence and Antimicrobial Resistance trends in Strains Isolated from Hospital in Italy.

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Background

Antibiotic resistance has become a main public health concern worldwide. Among the species with greater clinical interest, *Klebsiella pneumoniae* is a major opportunistic pathogen cause of urinary tract infections, pneumonia, and septicemia. An increased incidence of multi-drug resistant and extremely drug-resistant organisms of *K. pneumoniae* has been observed during the last decades in clinical practice. Therefore, the aim of the study was to identify the prevalence and trends of *Klebsiella pneumonia* antibiotic resistance from January 2015 to December 2019 at the University Hospital “San Giovanni di Dio e Ruggi d’Aragona” in Salerno, Italy.

Methods

Bacterial identification and antibiotic susceptibility were performed with VITEK 2. Statistical analysis was done using SPSS (IBM Corp, Armonk, NY). A chi-square test was used to compare the differences antibiotic sensitivities over the range of years considered in the study.

Results

A total of 3,157 isolates were collected from urine cultures, blood cultures, respiratory tract, and other samples. *K. pneumoniae* isolates showed the highest resistance rate to penicillin and cephalosporin antibiotic class, and lower resistance to fosfomycin and gentamicin. Extended spectrum beta-lactamase isolates were between 20-22%. Moreover, a low rate of resistance was shown for carbapenems class as imipenem (28%), meropenem (42%) and ertapenem (44%).

Conclusions

In recent years, *K. pneumoniae* infections have found worldwide importance, mainly due to multiple antibiotic resistances. Mainly the production of ESBLs and resistance to carbapenems is now a major public health problem. Constant monitoring of drug-resistant isolates is useful to find practical approaches to implementing antimicrobial therapy and reducing the spread of *K. pneumoniae* in nosocomial environments.

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249 - Identification of new antivirulence compounds active against cystic fibrosis Pseudomonas aeruginosa

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Identification of new antivirulence compounds active against cystic fibrosis *Pseudomonas aeruginosa*

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Introduction. Current treatments for *Pseudomonas aeruginosa* infections are becoming less effective due to the increasing rates of multi-antibiotic resistance, especially in cystic fibrosis (CF) patients. Pharmacological targeting of virulence through inhibiting quorum sensing (QS)-dependent virulence gene regulation has considerable therapeutic potential. In *P. aeruginosa*, the QS system regulates the production of multiple virulence factors and biofilm formation and, therefore, is a promising approach for developing antimicrobial adjuvants for combatting drug resistance. Here we report the hit discovery/optimization of quinazoline-based antivirulence compounds designed and synthesized based on the structure of endogenous modulators of PqsR, a key regulator of the QS system.

Materials and methods. Target quinazolines were synthesized by condensation of appropriately substituted anthranilic acids with acetic anhydride followed by treatment with hydrazine or with formamide to furnish the core bicyclic rings. The latter have been decorated through alkylation with a variety of heterocyclic and aromatic benzyl-derivatives. *P. aeruginosa* RP73, a prototype strain used for studying CF chronic pulmonary illness, was tested under conditions like those observed in the CF airways (artificial sputum medium, pH 6.8, 5% CO_2 atmosphere). MIC and MBC were measured by broth microdilution. Biofilm formation was evaluated in terms of biomass (extracellular matrix and cells, as assessed by crystal violet assay) and cell viability (viable cell count). Pyocyanin and pyoverdine formation was measured spectrophotometrically. The cytotoxic potential of each compound was evaluated toward IB3-1 bronchial epithelial CF cells by an MTS tetrazolium-based colorimetric assay.

Results. At 50 µM, some of the tested compounds affected biofilm formation, particularly NF3034 which reduced cell viability and extracellular matrix. Partial activity was shown by NF3017 and NF3033 (decreased cell viability), NF3077, and NF3081 (biofilm dispersion, although increased cellularity). More pronounced was the inhibition of pigments production. NF3072, NF3075, NF3069, and NF3088, affected both pyocyanin and pyoverdine formation. NF3033 and NF3086 affected pyocyanin only, while NF3077 and NF3081 were active against pyoverdine. Interestingly, none of the compounds showed antibacterial activity when tested in MIC assays or toxicity to IB3-1 cells at the same concentration used for the above assays.

Discussion and Conclusions. Through a panel of phenotypical assays, we highlighted some interesting antivirulence properties of quinazoline-based compounds against *P. aeruginosa* CF strain. The results so far obtained are of interest in the field of antibacterial drug discovery. Further studies to clarify the putative mode of action of the synthesized compounds are currently ongoing.

250 - Prevalence and Antimicrobial Profile of Causative Agents to Ocular Infections

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Prevalence and Antimicrobial Profile of Causative Agents to Ocular Infections

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Background

Bacterial ocular infections are a worldwide health problem and, if untreated, can damage the structure of the eye and contribute to permanent disability. Knowledge of the prevalence and antimicrobial susceptibility patterns of the main causative agents involved in ocular infections is necessary for defining an optimal antibiotic therapy. The aim of this study was to analyse bacterial species involved in ocular infections and the antimicrobial susceptibility patterns. Conjunctival swab samples were collected from patients with bacterial conjunctivitis at the University Hospital San Giovanni di Dio e Ruggi d’Aragona between January 2015 and December 2019.

Methods

Bacterial identification and antibiotic susceptibility were performed with VITEK 2. Statistical analysis was done using SPSS (IBM Corp, Armonk, NY). A chi-square test was used to compare the differences antibiotic sensitivities over the range of years considered in the study.

Results

A total of 281 causative agents of ocular infections were isolated, 81.8% of which were Gram-positive bacteria. Coagulase negative staphylococci (CoNS) were the most isolated species among Gram-positive bacteria, followed by *Staphylococcus aureus*. In contrast, *Pseudomonas* spp. and *Escherichia coli* were the main species isolated among Gram-negative bacteria (18.2%). Overall, linezolid, teicoplanin, tigecycline and vancomycin were the most effective antimicrobials. Analysis of resistance rates over time highlighted increasing resistance for azithromycin, clarithromycin and erythromycin among CoNS, and clindamycin and erythromycin among *Staphylococcus aureus*.

Conclusions

This study has identified the profiles of the major pathogens involved in ocular infection and their susceptibility patterns, which will help improve the treatments and the choice of antibiotics in ocular infections.

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253 - Antibiotic resistance profiles of microplastics associated microbial communities in the Adriatic Sea

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Antibiotic resistance profiles of microplastics associated microbial communities in the Adriatic Sea

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Introduction. Plastics have gradually become a global environmental pollution problem with a significant impact on human and animal health. Their large dispersion in the marine environment generates microplastics (MPs) which also release nanoplastics with effects on living organisms. The aim of this study was: i)to identify MPs and their associated microorganisms in the sea sediments of the Regional Natural Reserve Sentina (Adriatic Sea) and ii)to evaluate the antibiotic susceptibility patterns of the microbial communities.
Materials and Methods. Beach transects, parallel to the shoreline where the waves break, has been identified for the samplings. Each sample was in three replicas at three different positions of the transect (0, 15, and 30 meters). A protocol to perform microplastics isolation from sand sediments, suitable for biological samples and based on plastic floating in high salinity water, has been optimized. On floating microplastics, DNA isolation was performed with DNeasy PowerSoil Pro Kit (QIAGEN) for the shotgun metagenomic analysis. Isolated aerobic and anaerobic cultivable microorganisms have been identified by MALDI-TOF MS (Bruker). Susceptibility to a panel of 14 human and veterinary antibiotics, belonging to 12 different categories, was assessed by Kirby-Bauer and E-test methods.
Results. From sea sediments, the increased seawater density allowed plastics floating and through filtration, different kind of plastic materials, such as polyethylene, polypropylene, poly-methyl acrylate and poly-vinyl chloride were found. The metagenomics results are currently under processing and analysis. By microbial cultivation and MALDI-TOF MS identification, different bacterial species (n=29), both Gram -ve (55.2%) and Gram +ve (44.8%), have been isolated from the samples enriched in microplastics. Some bacteria are promising for the involvement in plastic degradation, such as *Lysinobacillus fusiformis*, *Exiguobacterium* sp., *Pseudomonas oleovorans*. Other bacteria are potentially pathogens, like *Clostridium septicum*, *Clostridium novyi*, *Shewanella putrefaciens*. Only the 17.2% of bacteria, *Exiguobaterium* sp., *Halomonas aquamarina*, *Paenibacillus brevicompactum*, *Lactobacillus fuchuensis*, *Providencia rettgeri*, resulted susceptible to all human and veterinary antibiotics. Higher percentages of resistance were observed for penicillins (85.7%) and monobactams (80.9%), in Gram +ve and Gram -ve, respectively. Althought not significant, a resistant pattern was recorded for carbapenems in Gram +ve (28.6%) and Gram -ve bacteria (61.9%, P=0.0532). Significant higher resistances for tetracyclines (64.3%, P=0.0166) were observed for Gram +ve microorganisms.
Discussion and Conclusions. The MPs may work as a vehicle of phenotypic resistant microorganisms in the Adriatic Sea environment.

255 - A high-resolution melting PCR assay for rapid detection of linezolid-resistance-associated new mutations in Mycobacterium avium complex

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A high-resolution melting PCR assay for rapid detection of linezolid-resistance-associated new mutations in *Mycobacterium avium* complex

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Background: *Mycobacterium avium* complex (MAC) is a group of slowly growing non-tuberculous mycobacteria that is emerging as major infectious threat in developing countries. The most frequent are *M. avium* and *M. intracellulare,* for which the conventional treatment is a macrolide-based multidrug regimen. However, due to the increased macrolide-resistance, alternative treatments are needed.
Linezolid, the only FDA-approved oxazolidinone for mycobacterial diseases representing 2nd line treatment for *M. tuberculosis,* has been shown to have a good activity against Non-Tuberculous *Mycobacteria* (NTM) [Winthtrop et al. 2015]. However, the long-term treatment, typical for mycobacterial infections, promotes the acquisition of linezolid-resistance due to mutations in *rrl*, *rplC* and *rplD*.
In this study we developed a High-Resolution Melting (HRM) PCR assay to detect linezolid-resistance-associated mutations in MAC.
Materials/methods: An HRM PCR assay was performed on 60 linezolid-resistant MAC clinical isolates (*55 M. avium* and 5 *M. intracellulare*) provided by ASST Grande Ospedale Metropolitano Niguarda (selected following American Thoracic Society Criteria) to detect linezolid-resistance-associated mutations in *rrl*, *rplC and rplD* genes.
The results were analysed using Precision Melt Analysis™ software (BioRad) obtaining different clusters based on mutations presence and then verified through Sanger sequencing.
Results: Preliminary results, based on 10 MAC (5 *M. avium* and 5 *M. intracellulare)*, showed in 20% of isolates mutations in *rplD gene*: G443A (Arg148Lys) in *M. intracellulare* and A439G (Thr147Ala) in *M. avium,* respectively**,** already identified in a recent study [Kim et al 2018]. We have also found new mutations, never reported before (data not shown) and under confirmation.
Conclusions: In recent years, in the Milan macroarea, MAC clinical isolates have shown low resistance to first line drugs (1.2% for clarithromycin and 4,7% for intravenously-administered amikacin), but high level of resistance for second line drugs (40.2% for moxifloxacin and 91% for linezolid). Linezolid-resistance is higher in our strains compared to that described in other parts of the world.
The HRM PCR assay is a rapid screening method to discriminate wild type from mutated sequences thanks to creation of different clusters. This can be useful to detect new and known linezolid-resistance-associated mutations both before and during treatment to monitoring the resistance onset.

258 - Biofilm formation, genetic signatures, and antimicrobial resistance profile of Neisseria gonorrhoeae strains

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Biofilm formation, genetic signatures, and antimicrobial resistance profile of *Neisseria gonorrhoeae* strains

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Introduction *Neisseria gonorrhoeae* is able to produce biofilms thus might be considered as a factor leading both to persistent of gonorrhea infections as well as influence the antimicrobial resistance profile. This study aims to evaluate the relationship between biofilm-forming capacity, the presence of some specific genetic signature by whole-genome sequencing (WGS), and the antimicrobials resistance profiles in *N. gonorrhoeae*. **Materials and Methods** A sample of 22 gonococci was analysed together with 5 World Health Organization (WHO) reference strains. The Biofilm Ring Test (BRT) method was used to quantify the biofilm production of *N. gonorrhoeae in vitro*, the attachment and initial biofilm

formation in the presence of DNase, and to assess the kinetic of early biofilm formation. The phenotypic characterization of gonococci was assessed by gradient diffusion methods (E-Test and MIC Test Strip) to define the antimicrobial susceptibility profiles. The genome sequencing was performed by Illumina NextSeq 500 platform, and the genomes were analyzed on https://pubmlst.org/organisms/neisseria-spp/. **Results** Strong biofilm producers (SBPs) were 71.4%, while 28.6% were classified as weak biofilm producers (WBPs). The cut-off for SBP classification was defined as three standard deviations above the mean BRT values obtained for the *N. gonorrhoeae* ATCC 49226 reference strain. In addition, DNase I treatment caused a significant ($P < 0.001$) reduction compared with proteinase K in the initial microbial attachment and biofilm formation. This suggests that biofilm formation in *N. gonorrhoeae* depends more on extracellular DNA (eDNA) than on protein production. Among SBP, four gonococci showed a decreased susceptibility to cefixime and resistance to ciprofloxacin. The majority of them belong to genogorup (G)1407 and to the *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) ST90 and Multi Locus Sequence Type (MLST) ST1901. The core genome MLST (cgMLST) scheme, which utilizes a set of 1649 loci, highlighted a high genetic diversity of gonococci. By genomic analysis of the target genes involved in biofilm production, we observed that the allele type 12 of the *nagZ* gene, which is involved in gonococcal biofilm disassembly, was predominant among the WBP producers. **Discussion and Conclusions** The results represent a first step beyond the state-of-art of antimicrobial resistance providing a novelty in the evaluation of *N. gonorrhoeae* resistant isolates.

260 - Dissection of the enigmatic role of Everolimus during Mycobacterium tuberculosis infections.

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Dissection of the enigmatic role of Everolimus during *Mycobacterium tuberculosis* infections.

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Introduction: Tuberculosis (TB) remains one of the most alarming infectious diseases worldwide. Although anti-TB treatment has a high efficacy in susceptible TB, the emergence and spread of resistant *Mycobacterium tuberculosis* (*Mtb*) strains is drawing attention to the development of new drugs and therapeutic strategies.
The search for new solutions includes the investigation of both novel agents and repurposing of approved drugs with potential anti-mycobacterial effects. A common trend is to consider host factors essential for pathogen survival or emergence of disease, focusing on both host metabolism and immunomodulation. All drugs that act mainly on the host are identified as host directed therapies (HDTs). Among the proposed molecules is everolimus, a compound that exerts an immunosuppressant activity by inhibiting the mTOR pathway and blocking growth-factor driven T-cell proliferation that has been described to exert both anti-mycobacterial and immunomodulatory activities
Even though everolimus has been investigated in a phase II randomized trial as a host directed therapy to treat TB, an oncological patient treated with everolimus for a neuroendocrine pancreatic neoplasia developed active TB twice and a non-tuberculous mycobacterial (NTM) infection in a time span of a year and a half.
Materials and Methods: To investigate this interesting case, we isolated and genotypically characterized the *Mycobacterium tuberculosis* (*Mtb*) clinical strain and tested the effect of everolimus on its viability in an axenic culture and in a human Peripheral Blood Mononuclear Cells (PBMCs) infection model. To exclude strain-specific resistance, we tested the activity of everolimus against *Mtb* H37Rv reference strain and clinical strains of ancient (EAI Manila) and modern lineages (H3). Furthermore, we investigated the proposed biological antimycobacterial mechanisms of everolimus, by studying its effect on ROS and autophagy induction during *Mtb* infection.
Results: Everolimus, and its analogues, did not show a direct effect on mycobacteria viability compared to isoniazid treated cultures. In PBMCs infection model, everolimus induced a negligible effect during *Mtb* infection in host cells only against *Mtb* H37Rv reference strain, but not against clinical isolates despite stimulating autophagy and a non-significant increase in ROS production was observed.

Discussion and Conclusions: With this case we want to underline that despite the need for therapeutical advances in TB treatment, a careful approach for testing, in relevant models, host directed therapies is necessary, as biological plausibility does not always translate to a pharmacological effect.

266 - The mobilome of probiotic Lactobacillus crispatus M247 includes Tn7088 a novel integrative and mobilizable element (IME) carrying a biosynthetic gene cluster for a class I bacteriocin

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The mobilome of probiotic *Lactobacillus crispatus* M247 includes Tn*7088* a novel integrative and mobilizable element (IME) carrying a biosynthetic gene cluster for a class I bacteriocin

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Introduction: *Lactobacillus crispatus* is the most frequently isolated species among the lactobacilli of a healthy human vaginal microbiome, however, not much is known about the *L. crispatus* mobilome. Here we report the complete genome sequence and the mobilome of the *L. crispatus* M247, a probiotic strain originally isolated from the stools of a newborn.
Materials and Methods: M247 genome was determined combining Nanopore and Illumina sequencing technologies and analyzed with bioinformatic tools. Mobile genetic elements were manually annotated using the NCBI and pfam databases and a functional analysis was carried out by quantitative real-time PCR.
Results: M247 genome consists of a single circular contig of 2,336,109 bp. M247 mobilome accounts for 14% of the whole genome and includes i) Tn*7088*, a novel 14,184-bp long integrative and mobilizable element (IME), ii) phiM247, a novel 42,649-bp long *Siphovirus* prophage, iii) 3 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) loci, iv) 226 Insertion Sequences (ISs) belonging to 15 families. Tn*7088* contains 16 ORFs and is organized in a mobilization module with relaxosome encoding genes, an integration/excision module and an adaptation module homologous to the listeriolysin S locus of *Listeria monocytogenes* encoding a class I bacteriocin. Comparison with Tn*7088*-like elements integrated in the genome of other *L. crispatus* strains available in the NCBI database, indicated that the adaptation module is subject to insertions and deletions mediated by ISs elements. PCR experiments showed that Tn*7088* element excises from M247 chromosome and produces a circular form, where the left and right ends are joined by *att*Tn, at a concentration of 3.92×10^{-5} copies/chromosome, restoring the *att*B insertion site (1.03×10^{-4} copies/chromosome). The prophage contains 52 ORFs, including genes for phage structural proteins, DNA replication and packaging, lysogenic and lytic cycle related proteins. Circular forms of phiM247 were present at a concentration of 3.40×10^{-5} copies/chromosome, whereas reconstituted *att*B sites were at 2.52×10^{-5} copies/chromosome.
Discussion and Conclusions: In this study, the mobilome of the probiotic *L. crispatus* strain M247 was defined by whole genome sequencing. M247 mobilome accounts for 14% of the genome and includes i) the novel IME Tn*7088* which contains the genes for relaxosome and encodes a functional integration/excision system as demonstrated by the formation of circular intermediates and ii) the novel prophage phiM247 encoding for a putative *Siphovirus* phage, which is able to excise from bacterial chromosome producing circular forms. Tn*7088* represents the first example of an IME carrying a bacteriocin biosynthetic gene cluster in *L. crispatus*.

267 - Epidemiological trends in Nontuberculous Mycobacteria isolation in the period 2018-2021 in a large tertiary care hospital

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Epidemiological trends in Nontuberculous Mycobacteria isolation in the period 2018-2021 in a large tertiary care hospital.

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Background: Despite decreasing prevalence in tuberculosis, the incidence of the diseases associated with non-tuberculosis mycobacteria (NTM) has been on the rise in Italy and around the world. The present retrospective study was conducted to determine longitudinal changes in the epidemiology and distribution of NTM over 3 years at tertiary care hospital Agostino Gemelli University Policlinic.

Meterials and Methods: We retrospectively analysed data on *Mycobacterium* species over 4 years (January 2018 to December 2021) by utilizing the laboratory information system. *Mycobacterium* species were identified using molecular tests and sequencing of hsp65 gene.

Results: We have detected a total 1100 samples positive for mycobacteria and, of these, we isolated in culture 112 NTM. The proportion of NTM continuously increased over these 4 –year period, from 3.77% in 2018 to 21,47% in 2021. Among the most common species were *Mycobacterium avium* complex (33.6 %), *Mycobacterium chimaera* (27,7%), *Mycobacterium intracellulare* (19,8 %), *Mycobacterium fortuitum* Complex (5,9%), *Mycobacterium* xenopi (3,96%), *Mycobacterium gordonae* (2,97%), *Mycobacterium kansasii* (2,97%), *Mycobacterium abscessus* (1,98%), and *Mycobacterium chelonae* (0,98%). In patients over 60 years, the proportion of NTM among the isolated increased from 10,89 % in 2018 to 36,63 % in 2021. Particularly the case *M. chimaera*, the isolation is 4 times more in 2021 than in 2018. The frequency and specie identification of these NTM have been associated with the clinical features of the patients, to assess the role of these mycobacteria in pulmonary and non-pulmonary diseases.

Conclusion: The number of NTM isolated continuously increased over study period. The role of *M. avium subsp. hominissuis* as a cause of pulmonary disease is a cause of concerns in the adult immunocompetent population.

268 - Shaping the subway microbiome by a probiotic-based sanitation during the COVID-19 emergency

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Shaping the subway microbiome by a probiotic-based sanitation during the COVID-19 emergency

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Introduction: The COVID-19 pandemics has highlighted how much the public transportation environment, such as subways, may be important for transmission of potential pathogenic microbes among humans. For these reasons, sanitation procedures including massive use of chemical disinfection were mandatorily introduced during the SARS-CoV-2 emergency, which however might worsen the antimicrobial resistance (AMR) threat, due to the reported favouring action of chemical disinfectants. By contrast, a probiotic-based sanitation (PBS) was recently shown to provide effective and long-term anti-SARS-CoV-2 activity and control of pathogens and AMR spread. Based on these observations, our aim was to assess its applicability and impact on the surface microbiome of the subway environment in comparison with chemical disinfectants during the COVID-19 pandemics.
Material and Methods: Two underground trains were enrolled in the study: one train served as control and continued to receive a conventional chemical sanitation, whereas the second train received the PBS in substitution of chemical disinfection. The presence of SARS-CoV-2 was monitored by droplet digital PCR (ddPCR), and the microbial contamination was evaluated simultaneously by conventional culture-based CFU count and molecular assays, including 16S rRNA NGS for bacteriome characterization and microarrays for the assessment of the microbial resistome.
Results: PCHS usage was associated with a stable >80% reduction in bacterial/fungal pathogens compared to the levels detected with chemical disinfection ($P < 0.01$), accompanied by a strong decrease of AMR genes ($Pc < 0.01$), as well as of SARS-CoV-2 presence ($P < 0.01$).
Discussion and Conclusions: The data presented here provide the first direct assessment of the impact of different sanitation procedures on the subway surface microbiome, allowing a better understanding of its composition and dynamics, and showing that a biological ecosustainable sanitation approach may be highly effective in counteracting pathogens' spread in our increasingly urbanized and interconnected environment without impacting on environmental pollution and further AMR diffusion.

269 - Peptidoglycan analysis of Streptococcus pneumoniae serotype 19A isolates belonging to the same clonal complex with different susceptibility to beta-lactams in the presence or absence of cefotaxime

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Peptidoglycan analysis of *Streptococcus pneumoniae* serotype 19A isolates belonging to the same clonal complex with different susceptibility to beta-lactams in the presence or absence of cefotaxime

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1. Introduction: *Streptococcus pneumoniae*, the pneumococcus, is a clinically relevant human respiratory pathogen responsible for more than a million deaths per year worldwide. Beta-lactam antibiotics have been employed to treat infections caused by *S. pneumoniae* for decades. However, beta-lactam resistant *S. pneumoniae* strains, frequently expressing multiple antibiotic resistance phenotypes, have increased dramatically since the 1980s and pose serious problems in the treatment of infections. The Hungary^{19A}-6 clone, a multiple antibiotic-resistant strain with unusually high-levels of beta-lactam resistance was prevalent in Hungary during the 1990s. This clone spread also in the Czech Republic and Slovakia but not significantly to other areas. In this work, we analyzed the peptidoglycan (PG) composition of two serotype 19A strains from Hungary, strains Hu15 and Hu17 as well as of two Hu15 derivatives in which the mosaic *pbp2x* and *pbp2x* together with *pbp1a* were introduced. Both Hu15 and Hu17 isolates are members of the same clonal complex, ST226, a single-locus variant of the representative strain HUN663 of the Hungary^{19A}-6 clone. Hu17 exhibits high-level penicillin resistance, whereas Hu15 is penicillin sensitive. This unique situation allows to study the development of beta-lactam resistance and to understand the impact of the main resistance determinants on the PG composition and, ultimately, on the resistant phenotype. 2. Materials and Methods: The PG of four *S. pneumoniae* isolates was extracted from culture grown in the presence or the absence of the beta-lactam cefotaxime and its composition was analyzed by high-performance liquid chromatography (HPLC) and mass spectrometry (MS). Growth and viability under the same conditions were also determined. 3. Results: Compared to the laboratory strain R6, the Hu strains had a PG profile showing an enrichment in branched *vs* linear peptides, consistent with the presence of a mosaic *murM* allele that is responsible for the synthesis of branched muropeptides and complex dimers, mostly bound through Ala-Ala crosslinks. These indirect cross-links are necessary for beta-lactam resistance but not sufficient for beta-lactam resistance in the absence of the mosaic PBP determinants. Moreover, as expected, the fraction of pentapeptides increased in all strains upon cefotaxime treatment in comparison to the condition of growth in absence of antibiotic. Finally, a bacteriostatic effect was observed upon treatment of the Hu strains with 1× MIC of cefotaxime with different behaviour depending on the specific strain. **4. Discussion and conclusion:** The results support that the Hu strains change their PG composition upon cefotaxime treatment, including increasing their cross-linked muropeptides and increasing fraction of pentapeptides, supporting the notion that cefotaxime selectively binds PBP2x and PBP3. Our results also show that beta-lactam resistance in the pneumococcus is a complex event that involve modifications in factors other than PBPs and they highlight that a specific genetic background is required to express full resistance.

270 - Antibiotic resistance pattern of Staphylococcus spp isolated from blood culture of hospitalized patients during the first and second wave of SARS-Cov2 infection: a single center retrospective analysis

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Antibiotic resistance pattern of Staphylococcus spp isolated from blood culture of hospitalized patients during the first and second wave of SARS-Cov2 infection: a single center retrospective analysis from Southern Italy.

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Introduction: The Sars Cov-2 pandemic infection has showed up old and new issue in health care system. Recent studies reported an increasing of blood stream infection (BSI) incidence in community as well as in hospital healthcare system. Moreover, due to abuse of antibiotics during the Sars Cov-2 pandemia, the increase of phenomena of antimicrobial resistance was salso observed. In our geographical area the impact of Sars Cov-2 infection in hospital setting has showed a difference of management between the first and second wave of infection with low incidence during the period June - October 2020. Therefore, to study Gram positive pattern of BSI in patients with and without Sars Cov-2 infection we retrospectively analyzed *Staphylococcus* spp isolation during the first and second Sars Cov-2 wave.
Materials and Methods: This study was a retrospective investigation of blood culture (BC) positive samples for *Staphylococcus* spp detected in 177 adult patients hospitalized for more than 48 hours at the Sant'Elia hospital in Caltanissetta from January 2018 to October 2020, of which 46 infected with Sars Cov-2.The samples for the blood cultures were collected aseptically by peripheral venipuncture from patients with a suspected bloodstream infection according to the guidelines.
Bacterial identification and antimicrobial susceptibility testing were carried out using the Vitek-2 System (BioMérieux, Marcy l'Etoile, France). According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, the antimicrobial sensitivity test of the strains was determined.
Results: Our results showed a significant most frequent infection correlated to *Staphylococcus aureus* in both group (Sars Cov-2 positive and negative), respectively 69,6% and 64,9%. Among the *Staphylococcus* spp the most significant presence was associated to *Staphylococcus capitis*, and *Staphylococcus hominis* for both two groups. In addition by comparison between two groups, we observed a significant presence of male gender in no Sars Cov-2 patients, while in Sars Cov-2 infected patients the mortality rate for BSI was more relevant.
Conclusions: The current study results showed the increasing of *Staphylococcus* spp, resistance, which may become a severe health-related issue in the future. Therefore, strict compliance of antibiotic use and employment of antibiotic stewardship programs at the public or private institutional level are recommended.

271 - Novel strategy against Chlamydiae: EVOO-based extracts.

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Novel strategy against *Chlamydiae*: EVOO-based extracts.

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1. Introduction

Extra virgin olive oil (EVOO), a cornerstone in the Mediterranean diet, is well-known for its nutritional and health properties, and, recently, has been increasingly studied for its anti-bacterial properties. To date, no studies have been performed on its activity against obligate intracellular bacteria, like *Chlamydia trachomatis*, leading cause of bacterial sexually transmitted diseases, and *C. pneumoniae*, responsible for respiratory infections, like pneumonia. In the last decades, clinical treatment failures to antibiotics have been reported, and, hence, novel therapeutic approaches need to be investigated. Therefore, here we evaluated, for the first time, the anti-chlamydial activity of a green EVOO-based extract in natural deep eutectic solvent (NaDES), as well as of its main polyphenol components, namely purified oleocanthal and oleacein in NaDES.

2. Materials and Methods

Different concentrations of EVOO extract (170 μg/mL, 17 μg/mL and 1.7 μg/mL), oleacein and oleocanthal in NaDES (55 μg/mL, 5.5 μg/mL and 0.55 μg/mL) were tested against *C. trachomatis* serovar D strain UW3 and *C. pneumoniae* strain IOL-207, in different phases of their growth cycle: i. pre-treatment of chlamydial Elementary Bodies (EBs); ii. pre-incubation of host cells; iii. treatment of chlamydial infected cells.

3. Results

EVOO extract in NaDES (170 μg/mL) showed the highest anti-chlamydial activity against *C. trachomatis* and *C. pneumoniae* EBs (decrease of the number of IFU/field 93.5% and 91.1%, respectively, $p<0.01$). Oleacein and oleocanthal (55 μg/mL) also showed efficacy ($p<0.05$) during the pre-treatment of chlamydial EBs. No anti-chlamydial activity was observed following pre-incubation or treatment phases.

4. Discussion and Conclusions

The effect of EVOO extract, as well as of oleacein and oleocanthal, in NaDES, against *C. trachomatis* or *C. pneumoniae* EBs, suggests EVOO as potential preventive strategy for reducing *Chlamydiae* infections.

272 - A new indole derivative dry powder for inhalation inhibits Staphylococcus aureus biofilm formation in vitro

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A new indole derivative dry powder for inhalation inhibits *Staphylococcus aureus* biofilm formation *in vitro*

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Introduction: Recent estimates suggest that biofilms account for over 80% of microbial infections. Biofilms can lead to life-threatening lung infections especially in patients with chronic respiratory diseases, including cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). In particular, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the two most common pathogens associated with chronic lung infections in CF patients. Effective treatment of biofilm-associated lung infections is hindered by the required high local drug concentrations. In this regard, inhalation is gaining attention as a valid strategy to increase lung drug concentration, reducing the potential side-effects associated with high doses and long-term administration regimens delivered by systemic routes. The aim of this work was to assess the effect of a new indole derivative dry powder on *S. aureus* biofilm formation and disgregation. **Materials and methods:** The compound 1-methyl-3-[6-(4-nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl]-1H-indole **(**SC38) was encapsulated into previously optimized chitosan microparticles (CS MP). The effects of SC38 and SC38-loaded CS MP was assessed on methicillin-sensitive *S. aureus* (MSSA) ATCC 6538 and methicillin-resistant *S. aureus* (MRSA) ATCC 43300 biofilm formation and disruption. Biofilm biomass and metabolic activity were determined through the crystal violet staining method and the XTT reduction assay, respectively. **Results:** The SC38-loaded chitosan microparticles exhibited favorable aerodynamic properties. SC38 was able to inhibit both the MSSA and MRSA biofilm formation in a dose-dependent manner, eliciting BIC_{50} of 3.7 and 8.0 µg/mL, respectively. The optimized formulation significantly reduced both MSSA and MRSA biofilm formation at all (for MSSA) and the highest (for MRSA) tested concentrations. No effect on preformed biofilm was observed. SC38-loaded CS MP showed a relatively safe profile in lung cells after 72 h exposure. **Discussion and Conclusions:** In this study, an inhalable dry powder formulation of the novel anti-biofilm compound SC38 was developed by spray-drying involving CS as a polymeric carrier. Our results

showed how this new indole derivative dry powder could be useful against biofilm-associated lung infections caused by *S. aureus*. Particularly, the proven efficacy in preventing biofilm formation is of great interest for the development of preventive therapies that aim to avoid the establishment of infections or to limit their progress. Future *in vivo* tolerability and efficacy studies are needed to confirm pre-clinical *in vitro* results and unravel the potential of this novel formulation for the treatment of difficult-to-treat biofilm-mediated lung infections.

279 - Uncomplicated UTIs and old antimicrobials: a retrospective study on southern Sardinian population

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Uncomplicated UTIs and old antimicrobials: a retrospective study on southern Sardinian population

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Introduction

Urinary tract infections (UTIs) are the most common among out-patient cases referred to microbiology labs. Therapy relies on a wide spectrum of available antimicrobials hence the most appropriate must be prescribed considering the species and their frequencies in the community, as well as possible emerging drug-resistant strains. In this regard, a broad panel of drugs, even dated, is still valid in terms of susceptibility scale such as nitrofurantoin, fosfomycin, cefepime and amoxicillin in combination with clavulanic acid. In addition, these drugs are cost-effective compared to the modern one; however are not free from side effects nor from contraindications, though limited by a careful anamnesis, physical examination and deep knowledge of the species mostly isolated in the reference community. Here, we focus on the species that are frequently isolated from UTI cases in the southern Sardinian region and their susceptibility to old antibiotics.

Materials and method

We performed a retrospective study by analyzing the reports from out-patient urine samples received and processed by our laboratory at the "Duilio Casula" University Hospital of Monserrato (Cagliari) between June 2021 and June 2022. Samples were seeded in linearcount and suspicious colonies isolated. Susceptibility data to nitrofurantoin and other common drugs such as fosfomycin, cefepime and amoxicillin/clavulanic acid were obtained by VITEK 2 compact for the different microbes, recorded and compared.

Results

Over a total of 574 samples, 455 tested positive to *E. coli* (79% of UTIs), *K. pneumoniae* (63), *E. faecalis* (25), *P. aeruginosa* (12), *C. koseri* (9), *P. mirabilis* (5), *E. aerogenes* (3), *P. stuartii* and *R. planticola* (1), respectively. The VITEK data regarding the most represented *E.coli*, showed it to be susceptible to nitrofurantoin (100%), cefepime (87%), fosfomycin (89%) and amoxicillin/clavulanic acid (71%).

Discussion and Conclusions

Beyond its affordability, nitrofurantoin seems to be the best choice for uncomplicated UTIs as suggested by the highest sensitivity of *E. coli* and *E. faecalis* although the high incidence of G6PD deficiency among Sardinians may represent a contraindication for its use. Fosfomycin still remains very advantageous for its bacteriostatic activity in single-dose. Amoxicillin/clavulanic acid shows a good percentage of susceptibility, however it is more expensive and may cause post-treatment dysbiosis. Finally, the more expensive cefepime is effective as fosfomycin but needs parenteral administration. Overall, these data suggest that the very cheap nitrofurantoin may be more convenient than other much more commonly used antimicrobials to treat most uncomplicated UTIs.

281 - Case report: Disseminated Cryptococcus neoformans in an immunocompetent patient.

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Case report: Disseminated *Cryptococcus neoformans* in an immunocompetent patient.

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Introduction: Cryptococcosis is an opportunistic fungal disease, caused by *Cryptococcus grubii*, *Cryptococcus neoformans* (*C. neoformans)*, and *Cryptococcus gattii*. These species mainly lead to lung infections and central nervous system infections, such as meningoencephalitis and cranial neuropathies. Other types of infections include skin, prostate, eyes, bone marrow and joints. Infection occurs more easily in patients with immunosuppression or with other infections. A very high mortality rate was reported for cryptococcal meningitis ranging from 27% to nearly 50%. We describe one case of meningoencephalitis from *Cryptococcus neoformans* that occurred in an apparently immunocompetent patient.
Materials and Methods: A 38-year-old Ukrainian woman was reported to be healthy up to 2 weeks before hospitalization, when she developed fever and confusion. After 48 hours, progressed to visual hallucinations along with urinary incontinence. The differential diagnosis included pulmonary tuberculosis, bacterial or fungal pneumonia, and lung cancer. The patient was subsequently diagnosed with disseminated *C. neoformans*, which remains very rare.
Results: A diagnosis of bacterial meningitis was made, and ceftriaxone and corticosteroids were given to reduce inflammation and edema of the brain and cranial nerves. Two days later, due to lack of clinical improvement, an aliquot of the cerebrospinal fluid (CSF) was tested, and it was found that she had a cryptococcal antigen title of 1: 320. Within 72 hours of culture, the laboratory notified doctors that *C. neoformans* was isolated from CSF. Using mass spectrometry (MALDI-TOF MS), the isolate was identified as subspecies *C. neoformans.* Therapy was changed to amphotericin B and her mental status improved significantly over the next 1 week and her cerebrospinal fluid culture had a decreased CSF cryptococcal antigen title to 1: 160.
Discussion and Conclusions: *C. neoformans* has a complex polysaccharide capsule with antiphagocytic properties. Low concentrations of anticryptococcal antibodies are normally found in immunocompetent people due to daily exposure. Although exposure is almost omnipresent in some regions of the world, this organism rarely causes clinically important infections in immunocompetent hosts. Conversely, it has become a notable opportunistic infection in those possessing a compromised cell-mediated immune response. Our case highlights the importance of collecting an accurate travel history in all patients, as the differential diagnosis should include atypical pathogens that may be endemic to the travel area. It also highlights the significant morbidity associated with cryptococcosis and drug-related toxicities and methods of preventing complications.

283 - Co-infection of Pseudomonas aeruginosa and Achromobacter xylosoxidans in green nail syndrome treated with ozenoxacin: a case report.

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Co-infection of *Pseudomonas aeruginosa* and *Achromobacter xylosoxidans* in green nail syndrome treated with ozenoxacin: a case report.

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Introduction Green nail syndrome (GNS) is a persistent greenish pigmentation of the nail plate, originally described in 1944 by Goldman and Fox, due to *Pseudomonas aeruginosa* infection. Recently, co-infection of *P. aeruginosa* and *Achromobacter* spp. have been described in patient with fibrosis cystic. *Achromobacter xylosoxidans* is an emerging, multidrug-resistant (MDR) pathogen involved in lung and soft tissue skin infection, widespread in nature, inhabiting mainly in water as *P. aeruginosa*. To data, no there are no recognized co-infection due to *P. aeruginosa* and *A. xylosoxidans* in skin and skin appendages. In this case report, we describe the first documented GNS due to *P. aeruginosa* and *A. xylosoxidans* co-infection.
Material and method A 65-year-old healthy retired woman become to our attention for a symptomatic greenish discoloration of the nail plate of the right and left fingernails over a period of 10 months. Dermatologic examination showed greenish-yellow bilateral discoloration of the first fingernail, in the proximal area, mild onychodystrophy of the entire nail plate, onychomadesis and distal onycholysis. Nail clipping was performed to confirm the clinical hypothesis of GNS.
Results Bacteriologic culture of nail clipping was positive for multidrug resistance (MDR) *A. xylosoxidans* and for quinolones intermediate resistance *P. aeruginosa*. Fungal coinfection was excluded by potassium hydroxide preparation and culture. Due to the co-infection of *P. aeruginosa* and *A. xylosoxidans*, topical therapy with ozenoxacin 1% daily for 12 weeks was prescribed. After the first 4 weeks of application, the patient presents clinical resolution, regrowth of the right nail and disappearance of the chloronychia of the left nail.

Discussion and conclusion The clinical management of the syndrome can be confusing, especially when the bacterial culture result is inconsistent or when non-*Pseudomona* bacteria are isolated with *P. aeruginosa*. In our case, due to the nail co-infection of *P. aeruginosa* and *Achromobacter spp.* no guidelines or previously reports have been published and antibiotic stewardship is crucial to act on both pathogens and reducing adverse events. However, for *A. xylosoxidans* there are no solid data regarding optimal topical antibiotic treatment. Ozenoxacin, the first nonfluorinated quinolone, could be a safe, topical treatment in case of MDR nail infections. This case highlights the complexity of the microbiological milieu in GNS and provide a possible treatment option in case of MDR co-infection of the nail unit. Further studies are required to evaluate clinical isolate from nail infections and the co-presence of *P. aeruginosa* and *A. xylosoxidans* as culprit bacteria in skin and skin appendage infection.

284 - Molecular characterization of Listeria monocytogenes strains isolated from food and human sources

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Molecular characterization of *Listeria monocytogenes* strains isolated from food and human sources

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Introduction: The aim of the work was the molecular characterization of *L. monocytogenes* (*L.m.*) isolated from food and humans in the period 2019-2021.
Materials and methods: A total of 67 *L.m.* isolates have been collected (UNI EN ISO 11290-1: 2017) at the Food Microbiology laboratories of the IZS Palermo from different food matrices with a prevalence of ready-to-eat food products such as dairy, fresh cheese, meat, fish sampled by Veterinary Services. The bacterial isolates were stored at -20 ° C in Microbank and sent to the LNR of Teramo for the determination of the serogroup (PCR-Multiplex) and molecular investigation (MLST and Whole Genome Sequencing -WGS). The sequencing was carried out on the Illumina NextSeq 500 platform and the data analysis was performed on a bioinformatics and data collection platform of the LNR *L.m*, called GenPat. N. 39 clinical strains of *L.m*. were collected at Palermo hospital laboratories (Regional Reference Laboratory for listeriosis). The strains, together with the sheets for the collection of epidemiological data, were sent to the Istituto Superiore di Sanità (ISS-Operational Microbiological Contact Point for L.m.) for molecular characterization by WGS. The sequencing was carried out on an IonTorrent S5 platform and the analysis was performed automatically on a bioinformatics and data collection platform, called IRIDA-ARIES.
Results: The results on the molecular characterization of the strains isolated from food showed a prevalence of serogroup IVb (70%), followed by IIa (24%), IIb (4.5%) and IIc (1.5%). The 46.3% of the strains, all belonging to serogroup IVb, have been isolated from milk products (mozzarella and string cheese) taken from the same producer during official controls, in the period August-September 2020, following a positivity detected in self-control. The results of the sequencing carried out by the LNR highlighted the existence of clusters of strains belonging to Clonal Complex (CC) 2, CC199 and CC1. In particular, all the strains isolated in the same cheese factory, both in the period considered and in a subsequent sampling in January 2022, belonged to CC2 and Sequence Type 2 (ST2). Regarding the 39 clinical *L.m.* strains, the phylogenetic analysis of the genomes allowed the identification of a Cluster_90 composed of 25 strains of which 6 were isolated in 2019, 16 in 2020, 1 in 2021 and 2 of which the year of isolation is unknown. The 25 strains were found to belong to serogroup IVb, ST2 and CC2, presenting between them an allelic difference in

the MLST core genome between 0 and 12. It has been noted the presence of 8 strains, isolated between October 2019 and August 2021, associated with maternal and neonatal cases. The genomic sequences of the 39 clinical strains of *L.m.* were compared with the sequences deposited in the IRIDA-ARIES database. From the analysis, it emerged that the Cluster_90, ST2 also included 3 isolated strains in Lombardy (2 in 2019, 1 in 2020), 2 in Piedmont, 1 in Lazio, 1 in Tuscany in 2020 and 2 isolated strains in Sicily (1 in 2019 from Syracuse and 1 in 2020 from the province of Palermo). Conclusion: This study allowed to start the construction of an integrated medical/veterinary network between laboratories to enhance the listeriosis surveillance system in the Sicily region.

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