ABSTRACT BOOK

Con la sponsorizzazione non condizionante di:

01 Patogenesi ed interazioni microrganismo-ospite

25 - Targeting PAD-mediated citrullination to treat human papillomavirus infections

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INTRODUCTION: Human Papillomaviruses (HPVs) are oncogenic DNA viruses that infect mucosal or cutaneous epithelia inducing cell proliferation. While most HPV types, referred as low-risk, produce mild benign pathogenic effects, a part of HPV types, called high-risk, may lead to cancer. 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 the molecular mechanisms regulating HPV-induced protein citrullination, we took advantage of different *in vitro* models of persistent, high-risk, HPV infections, *i.e.* HeLa and CaSki cells, containing integrated copies of HPV18 and HPV16, 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, as well as upon treatment with specific PAD-inhibitors (GSK-199, AFM30), indicating that citrullination is required for HPV pathogenesis. Consistently, p53 and p21, the main targets of HPV oncoproteins, are upregulated by PAD inhibitors. The overall citrullination nor the PADs profile are affected by E6 and E7 expression *in vitro*, but preliminary *in vivo* analyses revealed a significant association between PAD4 expression and cervical cancer progression.
DISCUSSION AND CONCLUSIONS: These findings could provide new insights into novel pathways elicited by high-risk HPV infection, which will constitute the rationale for the design of small molecule inhibitors able to block the specific factors responsible for PAD transactivation.

41 - From growth to virulence: how uropathogenic Escherichia coli (UPEC) reset gene expression during bladder cell adhesion

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Introduction: Urinary tract infections are a major concern in public health. The prevalent uropathogenic bacterium in healthcare settings is *Escherichia coli*. The increasing rate of antibiotic-resistant strains demands studies to understand *E. coli* pathogenesis to drive the development of new therapeutic approaches. **Materials and methods**: This study compared the gene expression profile of selected target genes in the prototype uropathogenic *E. coli* (UPEC) strain CFT073 grown in Luria Bertani (LB), artificial urine (AU) and during adhesion to host bladder cells by Reverse Transcription-PCR (RT-PCR) assays. **Results**: AU effectively supported the growth of strain CFT073 as well as other *E. coli* strains with different lifestyles, thereby confirming the appropriateness of this medium for in vitro models. Unexpectedly, gene expression of strain CFT073 in LB and AU were quite similar; conversely, during the adhesion assay, adhesins and porins were upregulated, while key global regulators were downregulated with respect to lab media. Interestingly, *papGII* allele was expressed in all tested conditions. **Discussion and conclusions**: Taken together, these results provide for the first time insights of the metabolic and pathogenic profile of strain CFT073 during the essential phase of host cell adhesion.

28 - 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 (RFs) 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 the IFN-gamma-inducible protein 16 (IFI16), a RF able to block HCMV DNA synthesis, can also counteract HCMV-mediated metabolic reprogramming in infected primary human foreskin fibroblasts (HFFs), thereby limiting virion production.

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 wild-type (WT) *vs.* IFI16 KO 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.

45 - Increased susceptibility to skin carcinogenesis and UVB-induced damage in immunodeficient human papillomavirus (HPV)-8 transgenic mice.

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Introduction: A large body of experimental and epidemiological evidence indicates that infection by skin-tropic papillomavirus belonging to the β-genus (□-HPV genotypes whose prototype is HPV8) contribute to increased skin cancer risk, especially in the immunocompromised setting, e.g. organ transplant recipients (OTR). Despite these findings, a causal role of these viruses has been difficult to verify because of their ubiquitous prevalence in the general population, their absence in some cancers, and the lack of experimental animal models. **Material and Methods:** To provide mechanistic insights into β-HPV-induced skin carcinogenesis in the immunosuppressed setting, a mouse model that recapitulates the events occurring in organ transplant recipient (OTR) was generated by crossing the β-HPV8 transgenic mice (K14-HPV8, that express the entire early region of the HPV8 genome in a skin specific manner) with Rag2 deficient mice (Rag2$^{-/-}$) that lack functional B and T lymphocytes. **Results and Discussion:** Our findings provide proof of concepts that immunosuppression accelerates HPV8-induced skin carcinogenesis. Indeed, skin cancer development in the Rag2$^{-/-}$: K14-HPV8 has been more aggressive in terms of incidence profile, lesion extension, and progression to overt cancer when compared to the immunocompetent counterpart, Rag2$^{+/+}$:K14-HPV8. As □-HPV-induced skin cancer occurs mainly in sun-exposed areas of the body with the contribution of UVB-induced DNA damage, we used our mouse model to demonstrate a causal link between HPV8 infection, immunosuppression, UVB exposure, and skin cancerogenesis. We show that exposure to a single low dose of UVB is sufficient to induce skin hyperplasia and inflammation in a remarkable short period of time in Rag2$^{-/-}$:K14-HPV8 while nothing relevant is happening in Rag2$^{+/+}$:K14-HPV8. UVB-induced DNA damage was still evident when the mice were sacrificed at 30 days post UVB-exposure as documented by immunohistochemical staining with the DNA-damage marker gamma-H2AX. A statistically significant accumulation of gamma-H2AX- positive cells was observed when UVB-irradiated Rag2$^{-/-}$:K14-HPV8 were compared to Rag2$^{+/+}$:K14-HPV8. Histologically, the skin of UVB-irradiated Rag2$^{-/-}$:K14-HPV8 was thicker, hyperplastic, and showed increased accumulation of mast cells in the dermis when compared to Rag2$^{+/+}$:K14-HPV8. Next, Bio-Plex immunoarrays have been used to compare the relative levels of 23 cytokines in skin extracts from Rag2$^{-/-}$:K14-HPV8 vs Rag2$^{+/+}$:K14-HPV8 mice that revealed an abundance of MCP-1, CXCL-1, IL-6, G-CSF, GM-CSF in the immunosuppressed mice when compared to the normocompetent counterpart.

5 - HLA-G AND CD147 EXPRESSION AS A SIGNATURE OF SARS-COV-2 PLACENTAL INFECTION

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Introduction: SARS-CoV-2 placental infection has already been reported, but no COVID-19 specific pattern of placental alterations, although the detection of fetal thrombovasculitis, villitis, and intervillositis as well as fetal and maternal malperfusion could be best interpreted as the signature of SARS-CoV-2 infection.

We selected Human Leukocyte Antigen-G (HLA-G) expression, an immunomodulatory molecule exploited as an immune-escape mechanism by several viruses, which is expressed during pregnancy and decreased in term placenta favoring childbirth, as a possible biomarker of placental infection. The aim of this study was to evaluate the differential SARS-CoV-2 infection in placental tissues, the effect on HLA-G expression and the possible role of SARS-CoV-2 receptors (hACE2 and CD147).

Materials and Methods: We evaluated placental tissue morphological aspects, SARS-CoV-2 Nucleoprotein (NP), HLA-G, hACE2 and CD147 expression in placenta samples from 18 asymptomatic and 10 symptomatic COVID-19 women and in 11 placenta samples from non-COVID-19 physiological term pregnancies enrolled at the University Hospital of Ferrara by immunohistochemical analysis. The expression levels for each antigen were evaluated by QuPath software analysis and reported as H-score and correlated with maternal and newborn clinical characteristics.

Results: COVID-19 placental samples showed increased inflammatory infiltrates associated to villitis and conversely a reduced decidual Natural killer cell presence compared to control group, mainly in symptomatic women. We found higher HLA-G expression in term placental tissues from symptomatic COVID-19 women positive for SARS-CoV-2 NP, mainly in villous trophoblasts (VT), in comparison with control term placenta samples. We reported a differential hACE2 and CD147 expression between symptomatic and asymptomatic COVID-19 placental specimens, with increased CD147 levels in the symptomatic cohort.

Discussion and Conclusions: We reported an increased SARS-CoV-2 NP expression in placental samples of symptomatic COVID-19 women, associated with morphological alterations and higher expression of HLA-G molecules and CD147 receptor in villous trophoblasts. Since HLA-G expression is involved in both immune regulation and vessel remodeling at the mother-fetus interface and a high CD147 expression might increase placenta viral susceptibility, we hypothesize a possible use of the levels and specific tissue localization of these biomarkers as signature of SARS-CoV-2 placental infection.

Funding: Unife crowdfunding, Unife COVID-19 grant.

Keywords: SARS-CoV-2, placenta, HLA-G, hACE2, CD147

134 - Analysis of Parvovirus B19 transcriptome in UT7/EpoS1 cells

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Introduction

Parvovirus B19 (B19V) is a human pathogenic virus responsible of a wide range of clinical manifestations. The ssDNA genome of 5.6 kb comprises a unique promoter directing the transcription of a pre-mRNA. A complex array of viral mRNAs is produced as a result of the diverse combinations of possible splicing and cleavage/polyadenylation events. The frequency of the processing events is different during infection leading to a two-state, early/late expression profile. The aim of this study is a detailed characterization of B19V transcriptome during a course of infection in UT7/EpoS1 cells through an advanced approach of Next Generation Sequencing (RNA-Seq).

Materials and Methods

B19V-infected UT7/EpoS1 cells were analysed at 0, 2, 16 and 48 hpi. Viral replication and transcription were assessed by qPCR e qRT-PCR, and capsid protein expression was observed by immunofluorescence. For RNA-Seq analysis, total RNA was extracted and purified from infected cells. Sequencing of RNA samples was performed on paired-end 150 bp mode on NovaSeq6000 (Illumina), by IGA Tehcnology Services (Udine). Downstream computational analysis was performed using the following tools: Trimmomatic 0.39, HISAT2 2.2.1 and StringTie 2.2.1 for trimming, mapping and counting the reads, respectively, and DESeq2 for the differential expression test.

Results

We observed an increase (+ 1 Log) of the amount of viral DNA and total RNA at 48 hpi by qPCR and qRT-PCR. Using different sets of primers, we detected an earlier appearance of non-structural (NS) protein mRNAs at 16 hpi, compared to the capsid proteins mRNAs that accumulated later in the course of infection. RNA-Seq analysis showed that 0.01% of the reads were correctly mapped on reference B19V genome at 16 hpi increasing to 0.5% at 48 hpi. Bioinformatics data confirm the prevalence of NS mRNA in the early stage of infection, while VP mRNAs were abundant in the late stage and revealed that cleavage at pAp2 site if preferred to the pAp1.

Discussion and Conclusions

Results confirm the existence of two-state transcription profile, ealy and late, during B19V infection in UT7/EpoS1 cells, allowing a precise mapping and fine characterization of the frequency of the diverse species of B19V mRNA. Future analysis with other cell types are necessary in order to investigate changes of the viral expression profile in different cellular environments. In addition, a deep investigation of the variation of the cellular expression profile during B19V infection could be useful to identify potential therapeutic target.

67 - SARS-CoV-2 infected pregnant women exhibit high transplacental passage of antibodies and dysbiotic vaginal microbiome

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Introduction: During the SARS-CoV-2 pandemic, pregnant women have been considered clinically vulnerable posing a major concern on how to handle the mode of delivery, the possibility of SARS-CoV-2 vertical transmission, and neonatal outcomes. Understanding the behavior of the virus during pregnancy is crucial for the maternal-infant dyad's health. **Materials and Methods:** Fifty pregnant women afferent to the Maternal and Child Hospital IRCCS Burlo Garofolo (Trieste, Italy) were enrolled in the study, divided into two groups: 30 pregnant women showing a positive nasopharyngeal swab for SARS-CoV-2 in the last trimester of pregnancy (COVID-19 group) and 20 pregnant women with a SARS-CoV-2 past infection acquired during the first or the second trimester of pregnancy (past-COVID-19 group). SARS-CoV-2 was tested in vaginal, rectal, placental and urine samples. The IgG positivity was evaluated in serum and funicular blood. In addition, vaginal and rectal microbiomes were profiled. Moreover, a nasopharyngeal swab from newborns was tested. **Results:** In COVID-19 group, four samples, including one vaginal, one rectal, one placental and one urine, tested positive for SARS-CoV-2. The presence of IgG was detected in 40% of sera and matched funicular blood. In the past-COVID-19 group, 47% of these samples tested positive. In the vaginal microbiome of the COVID-19 group, *Lactobacilli* decreased when compared to healthy controls from a pre-COVID-19 era (p=0.004). An heterogeneous vaginal microbial composition was distinctive of COVID-19 group with a high prevalence of *Bacteroides* and *Enterococcus faecalis*, identified also in rectal swabs, and of *Aerococcus*, a pathogen of urinary tract. In the vaginal and rectal swabs from the past-COVID-19 group, the beneficial *Phascolarctobacterium* was exclusively detected. One positive newborn was identified only in the COVID-19 group. No significantly neonatal adverse outcomes were observed in our cohorts. **Discussion and Conclusions:** This study confirmed that SARS-CoV-2 vertical transmission is a rare event, highlighting that maternal IgG immune response is efficient to block the passage of the virus to the fetus. The vaginal microbiome composition changed in the COVID-19 group, showing the translocation of opportunistic pathogens from the gut, as observed in women suffering with a vaginal dysmicrobism, driving an inflammatory response.

75 - Antimicrobial activity of Chitosan gel and deacetylated derivates.

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Introduction: Antimicrobial agents and their mechanisms of action are becoming important day by day. Chitosan is a partially deacetylated polymer of N-acetyl glucosamine. It is essentially a natural, water-soluble, derivative of cellulose with unique properties. Chitosan is usually prepared from chitin (2 acetamido-2-deoxy beta-1,4-D-glucan) that has been found in a wide range of natural sources like shellfishs and squids. One of the most investigated properties of chitosan is its antimicrobial effect embracing from biomedical to cosmetic area as well as from food to agriculture applications. For this reason, the aim of the present study was to evaluate the antimicrobial activity of chitosan-gel with its deacetylated derivatives at different concentration in clinical and references strains. **Materials and Methods:** The bacterial strains were grown overnight at 37°C in 30 mL of Tryptone Soya Broth (TSB). The pellet was washed with PBS1x and it was resuspended in10 mL of TSB and added to 90 mL of H_2O and a portion of this suspension was put in contact with autoclaved Chitosan gel, 91.5% Chitosan Deacetylated gel and 88.3% Chitosan deacetylated gel at different concentrations (0.5%, 1% and 1.8%) and solutions were incubated at 37°C. The bacterial viable count for each strain was evaluated at time zero (T0), 15, 30 minutes and 1, 2, 4, 24 hours after contact with the Chitosan gels. **Results:** As expected, the results showed a marked antimicrobial activity of the Chitosan gels against all the bacterial strains analyzed, both clinical and references strains. In addition, the bacterial action of 91.5% and 88.3% deacetylated chitosan gels is more aggressive than autoclaved chitosan gel. **Discussion and Conclusions:** The experimental data obtained in this work highlighted the strong antibacterial effects of deacetylated chitosan gel against all analyzed strains. In the near future it could be interesting to evaluate the effect of the interaction between Chitosan gels and other molecules that could enhance its bactericidal ability.

51 - Analysis of the interactions between Spheroids and bacterial cells.

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Introduction. An experimental model widely used to study tumor growth and invasiveness is today represented by 3D *in vitro* cultures such as Spheroids, constituted by aggregates of tumor cells. The advantages of this 3D model consist in the relative simplicity of Spheroids preparation, in the opportunity of using different cell lines, and the possibility to evaluate the effect of drugs and/or new compounds in a three-dimensional environment more similar to the physiological one than cell culture. The main objective of this study was to analyze the interactions between 3D *in vitro* tumor Spheroids and bacterial cells. These interactions are of considerable interest in cancer research, because have been found in many oncological pathologies characterized by a significant inflammatory component. **Material and Methods.** Firstly, starting from the NIH3T3 cell line (fibroblast deriving from mouse embryo), the Spheroids were obtained. The cells were seeded, in a coated agarose 48-wells, at various cell densities in order to verify the possibility to obtain the Spheroids at least 300-400μm in diameter (from $1x10^3$ to $7x10^3$ cells/well). The sphericity and the size of Spheroids under the microscope (Zeiss observer Z.1) were evaluated. Subsequently, the NIH3T3 Spheroids were infected with *Staphylococcus aureus* ATCC6538 and with methicillin resistant-*S. aureus* (MRSA) clinical isolate. **Results.** During the time of the observation the size, the sphericity and then the amounts of intra-Spheroids bacteria were evaluated. Moreover, to investigate and better understand the size of the necrotic area within the Spheroids a fluorescence assay was performed. Finally, the antibacterial activity of human Beta-HBD3 defensin analogue against staphylococcal strains was evaluated in Spheroids invasion assays. **Discussion and Conclusion.** These preliminary data lay the foundations for the development of a 3D infection model useful for understanding the infection/inflammation processes in complex environments. This project is aimed to evaluate novel compounds against multidrug-resistant bacterial infections in 3D *in vitro* model. Thanks to this technology we expect to give our contribution to precision medicine with ad hoc drugs.

59 - Assessment of dalbavancin effect on human phagocyte in vitro functions against multi-drug resistant Staphylococcus aureus

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Introduction: Acute bacterial skin and skin structure infections (ABSSSIs) are a significant global healthcare burden: the incidence and severity of these diseases increased in recent years, in parallel with the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA). Polymorphonuclear leukocytes (PMNs) are the most important cell type involved in the early nonspecific host response to bacterial pathogens, but the ability of *S. aureus* to subvert, evade, or tolerate immune responses contributes to its persistence and capacity to create various infections. Dalbavancin (DAL), a lipoglycopeptide, is indicated for the treatment of ABSSSIs, and has a broad spectrum of action against most microorganisms, including those resistant to other antimicrobials. Its structure has been altered to enhance activity against MRSA, to extend its half-life and to provide a convenient and well-tolerated treatment option. The aim of this study was to determine the straightforward performance of DAL upon the binomial antibiotic resistant staphylococci and host defenses, to establish its potential immunomodulating activity.
Materials and methods: The *in vitro* functional activity of human PMNs against MRSA was evaluated by testing, in absence or presence of DAL at different concentrations, various PMNs post-phagocytosis functional activities (i.e. intracellular killing, drug uptake, apoptosis, ROS production, cytokine release, etc.), before and after bacterial stimulation. To differentiate between any separate effect of DAL on the bacteria and PMNs, *in vitro* DAL pretreatment assays were also performed by the exposure of each of them to DAL (at 1xMIC) for 1 h, before they were incubated together.
Results: Our results evidenced in DAL presence an enhanced MRSA killing activity by PMNs in comparison with DAL-free controls, within 90 minutes of incubation. In fact, in control condition PMNs were able to scanty kill ingested MRSA, whereas with the addition of DAL, significantly ($p<0.05$) enhanced their staphylococcal killing. The assays with DAL pre-treated MRSA or PMNs highlighted a similar trend: an improved staphylococcal killing due to DAL direct effect on both staphylococci and PMNs within 90 minutes of incubation. In parallel, anti-inflammatory effect (mainly, decreased level of TNF-α), a slight increase into the oxidative burst, and a delay in neutrophil apoptosis were observed in PMNs in presence of both DAL and MRSA.
Discussion and conclusions: Of the infections with human pathogens that require neutrophils for control and clearance, infections with MRSA are particularly prevalent. A key virulence strategy used by many pathogens and by MRSA too is to subvert effective immune responses. In the present study, we highlighted that DAL acts in synergism with neutrophils by modulating both staphylococcal killing and cytokine release. A role of TNF in apoptosis regulation has been revealed that could indirectly influence the killing capacity. These preliminary results draw attention to the need of a deeper understanding of the mechanisms exerted by DAL on neutrophil functional activities.

34 - Herpes simplex virus type 1-induced citrullination in neuronal cells suggests new therapeutic options for the treatment of virus-associated neurodegenerative disorders

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INTRODUCTION. Herpes simplex virus type 1 (HSV-1) is a neurotropic virus, with a seroprevalence ranging from 60 to 90% in adults. The pathogenesis of HSV-1 infection follows a cycle of primary infection of epithelial cells usually in childhood, latency primarily in neurons, and reactivation. Recent evidence suggests that HSV-1 may be involved in the etiology of Alzheimer's disease (AD). Sporadic AD is a complex multifactorial neurodegenerative disease with evidence indicating coexisting multi-pathogen and inflammatory etiologies. Citrullination is a post-translational modification catalyzed by peptidyl arginine deiminases (PADs), whose dysregulation has been associated with AD and others neurodegenerative disorders. We have recently established that human cytomegalovirus (CMV), another member of the *Herpesviridae* family, triggers PAD-mediated citrullination and this activity promotes viral fitness. Moreover, we have also shown that PAD-inhibitors inhibit CMV and HSV-1 replication *in vitro*. Based on these premises, the goal of this project was to characterize the citrullination profile during HSV-1 infection in different neuronal *in vitro* models.
MATERIAL AND METHODS. Through real-time quantitative PCR (qPCR) and Western blot analyses, we investigated the PAD expression profiles during HSV-1 infection in human neuroblastoma and medulloblastoma cell lines (SHSY-5, ONS-76 and DAOY, respectively). Moreover, by using a well characterized citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe, the pattern of citrullination was tested in the same cellular models. Finally, we evaluated the antiviral activity of the permeable pan-PADs inhibitor Cl-amidine. To this purpose, and to carefully quantify viral gene expression and viral replication upon treatment, several techniques have been employed, including standard plaque assays, qPCR and Western blotting.
RESULTS. The results obtained so far clearly demonstrated that HSV-1 triggers PAD2, PAD3 and PAD4 isoforms expression, both at mRNA and protein levels. Interestingly, the overall citrullination profiles obtained with the RhPG probe changed consistently at different time points during infection in all the cell lines tested. Moreover, the inhibition of PADs significantly impaired the expression of viral genes and HSV-1 replication as well. Remarkably, this reduction was much more pronounced in the cell lines of neuronal origin, and in particular in SHSY-5Y cells, in comparison to human primary fibroblasts and Vero cells which were used as controls.
DISCUSSION AND CONCLUSION. Our findings provide new insights into the mechanism associated to HSV-1 replication in neuronal cells, supporting the rationale for innovative medical interventions for the treatment of neurodegenerative diseases, including AD.

38 - Novel antiviral activity of PADs inhibitors against human betacoronaviruses SARS-CoV-2 and HCoV-OC43

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INTRODUCTION. Emerging zoonotic RNA viruses have repeatedly attracted the attention of researchers over the past few decades. Novel coronaviruses (CoVs) in particular need special attention, due to the high mortality rates, the lack of effective therapies, the potential to spillover from a large reservoir of animal hosts, and the high rate of transmissibility that allows them to cause epidemics. To date, seven human CoVs (HCoVs) have been identified: among them, HCoV-OC43 and SARS-CoV-2, the causative agent of the ongoing epidemic of atypical pneumonia (COVID-19), belong to beta genus. A very recent study described the putative roles of a family of cellular enzymes called peptidylarginine deiminases (PADs) in COVID-19 disease. PADs are a family of calcium dependent enzymes that catalyze the post-translational modification citrullination, a process in which the guanidinium group of a peptidyl-arginine is hydrolyzed to form peptidyl-citrulline, a non-genetically coded aminoacid. PADs dysregulation leads to an aberrant citrullination which is a characteristic biomarker of several inflammatory conditions. Moreover, a correlation has recently emerged between PADs dysregulation and other viral infections, including human rhinovirus and cytomegalovirus. Based on these evidences, the aim of this work was to evaluate whether PAD inhibitors were a reliable new class of host-targeted antivirals against coronaviruses.
MATERIAL AND METHODS. By using the HCoV-OC43 and SARS-CoV2 strains as models of infection in human lung fibroblasts (MRC-5) and monkey kidney cells (Vero-E6), 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 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. HCoV-OC43 and SARS-CoV-2 infections were significantly associated to PAD-mediated citrullination *in vitro* and to an increase of PAD expression, both at mRNA and protein levels. Moreover, the pharmacological inhibition of PAD enzymes led to a significant reduction of viral replication, suggesting that PAD4 isoform in particular might play a major role in OC43 replication.
DISCUSSION AND CONCLUSION. Our results suggested that i) citrullination is a process that can be induced by RNA viruses, such as HCoV-OC43 and SARS-CoV-2, as a mechanism to foster their replication, and 2) that increase of PADs activity is central for beta-coronavirus replication. Taken together, we provide evidence that PADs inhibitors deserve consideration against human beta-coronaviruses infection.

85 - Redox-modulating compounds in the treatment of coronavirus and influenza virus infections

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Introduction: The generation of Reactive Oxygen Species (ROS) is a key event during respiratory virus infections, and it is responsible for the regulation of different steps of virus life cycle. Influenza virus (IV)-induced ROS production activates redox-sensitive pathways useful for its replication. About coronaviruses (CoVs), it has been observed that oxidative stress regulates also virus/host cell binding. Indeed, in SARS-CoV-2 model, oxidation of the cysteine residues on the peptidase domain of ACE2 receptor and RBD of the Spike protein, favors viral entry. Based on this evidence, we aimed at exploring the efficacy of redox-modulating compounds, against CoV and IV infections, by interfering with different steps of viral replication.

Materials and Methods: SARS-CoV-2 or influenza virus A/PR/8/H1N1 were pre-incubated for 1 h at 37° C with different thiol compounds: a GSH-precursor, a conjugate of N-acetylcysteine and s-acetyl-β-mercaptoethylamine, able to increase GSH content; its dithiol derivative; or the N-butanoyl GSH derivative (GSH-C4). The mixture was used to infect ACE2-expressing cell lines or A549 cell line. In parallel, the same compounds were also added 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 results showed that all the reducing agents were able to impair the infectivity of SARS-CoV-2. The virucidal assay showed that GSH-C4 treatment decreased plaque formation by 79±5%, the GSH-precursor and its metabolite by 88±2% and 95.5±0.7% respectively, suggesting that reducing conditions strongly impair the binding between the spike protein and its receptor. The use of GSH precursors also interfered with IV entry. Indeed, A549 cells infected with a mixture of IV and GSH-precursor showed a lower expression of viral proteins compared to untreated condition. Moreover, the same compounds, when added for 24 h post infection, were able to increase the antioxidant response and to inhibit IV replication.

Discussion and Conclusions: Redox-modulating compounds can act by two possible mechanisms: preventing the binding between viruses and host receptors or interfering with viral replication by the modulation of the antioxidant response in host cell. Further studies are in progress to clarify the effect of reducing compounds during the replication of SARS-CoV-2 and their properties in regulating redox-sensitive pathways important in the inflammatory response.

156 - SILVER NANOPARTICLES FROM CAMPANIA REGION RED GRAPE CULTIVARS (AGLIANICO, FIANO AND GRECO) AND THEIR ANTIVIRAL ACTIVITY

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Aim of study. Herpes simplex virus 1 (HSV-1) is a neurotropic virus that after primary infection of epithelial cells becomes latent in neurons of the peripheral nervous system and can be periodically reactivated resulting in recurrent clinical or subclinical episodes throughout life. Canine distemper virus (CDV) is considered as a highly contagious and an acutely febrile disease in dogs. In the prevention of epidemic emerging and neglected viral infections, alternative antiviral agents are need. In the last 20 years, nanotechonology have emerged as potential alternative antimicrobial agents and for drug delivery, both in vitro that in vivo models.

The interaction between silver nanoparticles and viruses is attracting great interest due to the potential antiviral activity of these particles. In this study, we demonstrate that stable, non-cytotoxic silver nanoparticles synthesized from bark extracts of *Aglianico, Fiano, Greco* (red grape cultivars of Campania region). We show that production of silver nanoparticles from different vine varities is feasible and capable of reducing viral infectivity of Herpes Simplex Virus (HSV) and Canine Distemper Virus (CDV), probably by blocking interaction of the virus with the cell.

Methods used. The bark extracts of *Aglianico, Fiano, Greco* were challenged with 1mM silver nitrate solution, silver nanoparticles formation leads to colour changes from colourless to brown with the excitation of surface plasmons. Silver nanoparticles were characterized with different techniques i.e. UV–VIS Spectroscopic Analysis, dynamic light scattering (DLS), Zeta potential Analysis, Fourier-transform infrared spectroscopy (FTIR) and transmission electron microscope (TEM). To evaluate cytotoxicity of nanoparticles MTT assay was performed based on the reduction of MTT to the insoluble and dark blue formazan by viable and metabolically active cells. Further, antiviral activity of AgNPs on HSV and CDV infectivity *in vitro* was used a co-treatment assay in which the different AgNPs of interest and viruses were concomitantly added to the cell culture in order to have both of them present during and after viral adsorption. For pre-treatment, virus was pretreated with the different AgNPs for 1 hour before infection. The extent of HSV and CDV replication was assessed by plaque titration after addition of carboxymethyl cellulose.

Results and conclusions. From the present study it was observed that AgNPs were capable of controlling viral infectivity, which might be dependent on characteristics of the AgNPs. It was observed that AgNPs produced by *Aglianico, Fiano, Greco* have a size of 57nm, 61 nm and 58 nm, respectively. The FTIR analysis showed presence of protein capping agent when AgNPs scanned 400-4000 cm^{-1}. Nanoparticles have considerable antiviral activity against HSV and CDV and were less cytotoxic on Vero cells. The synthesized silver nanoparticles may have important advantage over conventional antibiotics to which the viruses got resistance.

87 - Graphene nanoplatelet and graphene oxide functionalization of face mask materials inhibits infectivity of trapped SARS-CoV-2

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Introduction: Coronavirus Disease 2019 (COVID-19) is one of the biggest challenges of the 21st century. To ease the pressure on the healthcare systems, while waiting for better treatments against SARS-CoV-2 and vaccines, most countries introduced the mandatory use of face coverings. Early in the pandemic, in April 2020, the Centers for Disease Control and Prevention recommended the use of face masks in areas with high rates of transmission. Increased demand for protection against SARS-CoV-2 and other airborne pathogens, boosts the design and production of innovative solutions by industry stakeholders.
Nanoparticles, nanofibers, and other pioneering technologies based on nanomaterials have been introduced in mask production chains to improve performance and confer antiviral properties.
Recent advancements in bidimensional nanoparticles production such as graphene (G) and graphene oxide (GO) have the potential to meet the need for highly functional personal protective equipment (PPE) against SARS-CoV-2 infection. Engineered textile for use in PPE would satisfyingly limit COVID-19 spread thanks to the ability of G and GO to interact with microorganisms.
Currently used PPEs only provide a physical barrier that decreases infection likelihood and do not inactivate the virus.
Material and methods: SARS-CoV-2 incubated for two hours with GO was used to infect VERO cells. Viral replication and cell viability was monitored by using immunofluorescence and crystal violet staining. Additionally, G and GO was used to functionalize polyurethane or cotton fabrics that were then used in two different experimental settings to assay ability to reduce viral infectivity. We evaluated both filtration and static incubation with SARS-CoV-2 suspension.
Results: Our work shows that SARS-CoV-2 pre-incubation with soluble GO inhibits VERO cells infection. Furthermore, after SARS-CoV-2 exposure, fabric infectivity of G/GO-functionalized polyurethane or cotton was nearly nil.
Discussion and conclusion: These findings may significantly shape queries on innovative nanomaterial-based strategies not only for SARS-CoV-2 virus prevention, but also in water filtration, air purification, and diagnostic methods implementation.

61 - Phosphatidylcholine liposomes down-modulate CD4 expression reducing HIV entry in human type-1 macrophages

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Introduction. Despite the efforts and improvements in antiretroviral therapy, Human Immunodeficiency Virus type-1 (HIV-1) infection still remains one of the most public health emergencies. One of the strategies adopted to contrast HIV-1 infection is based on interfering with the virus entry into target cells, which occurs after the interaction between viral gp120 and CD4 receptor expressed on surface of target cell. CD4 down-regulation and consequent reduction in HIV-1 entry have been reported in the past in a manner which may be dependent by Protein Kinase C (PKC), which, in turn, may be activated by phosphatidylcholine (PC) through a number of mechanisms, involving different phospholipases. On these grounds, we evaluated the capability of PC liposomes to interfere with the initial stage of HIV-1 infection in human macrophages.
Materials and Methods. PC liposomes were tested on human lymphocytes and monocyte derived type-1 macrophages (M1) obtained from buffy coats of anonymized healthy donors. After stimulation, the expression of CD4 receptor and chemokine receptor type 5 (CCR5) on target cells was evaluated by flow cytometry. In order to investigate on mechanism of CD4 internalization and degradation, the surface and intracellular expression of CD4 receptor was evaluated by flow cytometry in the presence of different inhibitors and by evaluating the phosphorylated CD4 levels by means of Western Blotting. The efficacy of the treatment was analysed in term of reduction of HIV-1 entry in PC liposomes pre-treated M1.
Results. We found that PC liposomes reduced the expression of CD4 receptor in M1, but not in $CD4^+$ T cells. The observed down-regulation was specific for CD4, as any effect was not observed in CCR5 membrane expression. Moreover, the reduction of membrane CD4 expression required the involvement of Ca^{2+}-independent PKC, which in turn mediated serine phosphorylation at the intracytoplasmic tail of CD4 receptor. Serine phosphorylation of CD4 was also associated with its internalization and degradation in acidic compartments. Finally, the observed CD4 down-regulation induced by PC liposomes in human primary macrophages reduced the entry of a R5 tropic single cycle replication HIV-1.
Discussion and Conclusions. Altogether, these results show that PC liposomes may interfere with the first steps of HIV-1 infection in human M1, by inducing CD4 down-modulation, and may impact HIV pathogenesis by lowering viral reservoir.

4 - Strigolactone analogs are promising antiviral agents for the treatment of human cytomegalovirus infection

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The human cytomegalovirus (HCMV) is a widespread pathogen and is associated with severe diseases in immunocompromised individuals. Moreover, HCMV infection is the most frequent cause of congenital malformation in developed countries. Although nucleoside analogs have been successfully employed against HCMV, their use is hampered by the occurrence of serious side effects. There is thus an urgent clinical need for less toxic, but highly effective, antiviral drugs. Strigolactones (SLs) are a novel class of plant hormones with a multifaceted activity. While their role in plant-related fields has been extensively explored, their effects on human cells and their potential applications in medicine are far from being fully exploited. In particular, their antiviral activity has never been investigated. In the present study, a panel of SL analogs has been assessed for antiviral activity against HCMV. We demonstrate that TH-EGO and EDOT-EGO significantly inhibit HCMV replication *in vitro*, impairing late protein expression. Moreover, we show that the SL-dependent induction of apoptosis in HCMV-infected cells is a contributing mechanism to SL antiviral properties. Overall, our results indicate that SLs may be a promising alternative to nucleoside analogs for the treatment of HCMV infections.

107 - Complex electromagnetic fields reduce Candida albicans planktonic growth and its adhesion to titanium surfaces.

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Introduction: This study evaluates the *in vitro* effects of the exposure of *C. albicans* to different programs emitting pulsed Electron Magnetic Fields characterized by a remarkable harmonic richness, different intensity, frequency, duration and type of wave (Machines Codes), all of which accounts for the denomination of "complex magnetic fields, CMFs". To do this, planktonic cultures of *C. albicans* were exposed to CMFs and analyzed for viable count, metabolic activity, cell morphology and filamentation analysis. Then, considering the pathogenic role of *C. albicans* in oral cavity, the microorganism ability to adhere to titanium surfaces was evaluated after expositions to CMFs. **Materials and Methods**: *In vitro* cultures of *C. albicans* ATCC 10231 were exposed to different cycles and programs of electromagnetic fields defined as: Oxidative Stress, Oxidative Stress plus Antibacterial, Antibacterial, Antibacterial plus Oxidative Stress. Colony forming units (CFUs), metabolic activity (INT), cells viability (Live/Dead), cell morphology and filamentation analysis were performed to the outcome detections. Moreover, the broth cultures, exposed to the different CMFs, were grown on titanium discs for 48 h. The quantity comparisons of adhered *C. albicans* on surfaces were determined by cell count (CFUs) and visualized by scanning electron microscopy (SEM). **Results:** Microbiological analysis showed that the *C. albicans* growth could be readily controlled with CMFs reducing the number of cultivable planktonic cells *vs* controls, independently by the treatment applied. In particular, the antibacterial program was associated to lower levels of CFUs. The quantification of the metabolic activity through the INT assay was significantly lower by using the oxidative stress program. Live/Dead images showed that CMFs significantly decreased the amount of viable *C. albicans* cells. CMFs inhibited *C. albicans* virulence traits reducing both hyphal morphogenesis, adhesion and biofilm formation on titanium discs. **Discussion and Conclusions:** Independently of the adopted protocol, CMFs exert ability in the inhibition of planktonic growth, virulence transition into the filamentous form and reduction of capability to adhere to titanium surface of *Candida albicans*. Based on these results we can affirm that CMFs, at the used parameters, is a promising technology for potential applications in the treatment of *C. albicans* infections.

30 - Longitudinal, virological and serological assessment of hospitalized COVID-19 patients

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INTRODUCTION: Emerging evidences show that SARS-CoV-2 could be shed through multiple routes. Whether the virus can be detected in specimens from other sites, like feces and blood, and therefore potentially transmitted in other ways than by respiratory droplets, is unknown. The aim of this study was to describe the virological and serological assessment of COVID-19 patients hospitalized in Milan, Italy, during the first epidemic wave.
MATERIALS AND METHODS: Nasopharyngeal (NPS), anal swabs and blood samples were collected from 23 COVID-19 patients, at hospital admission, and periodically up to discharge, for a median time of 20 days (3-83 days). RNA was isolated and tested for SARS-CoV-2 by qRT-PCR; anti-SARS-CoV-2 IgM and IgG antibody titers were evaluated in serum samples by ELISA.
RESULTS: SARS-CoV-2 genome was detected in the NPS swabs of the 23 patients, at the admission, and 8/19 (42.1%) were still positive at the discharge. Anal swabs were positive to SARS-CoV-2 RNA detection in 20/23 (86.9%) patients; 6/19 (31.6%) were still positive at discharge. The mean time of RNA negative conversion was 17 days (4-36 days) and 33 days (4-77 days), for NPS and anal swabs, respectively. SARS-CoV-2-RNA was detected in the blood of 6/23 (26.1%) patients. Thirteen/23 (56.5%) and 17/23 (73.9%) patients were seropositive for IgM and IgG, respectively, at the admission, and the median IgM and IgG levels significantly ($p<0.05$) increased after 13 days.
DISCUSSION AND CONCLUSIONS: Our report provides evidence that SARS-CoV-2 is shed through multiple routes, with important implications in healthcare settings.

20 - Immune cell response to P. aeruginosa and S. epidermidis in biofilm and planktonic mode of growth suggests different strategies adopted by these two bacterial species in the interaction with the host

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Introduction: Despite the extensive biofilm-research carried out in the last decades, our understanding of the human immune response to microbial biofilms is still poor. There is evidence that the host immune response is only partially beneficial in clearing biofilm-associated infections if not even harmful. Indeed, the simultaneous activation of both the innate and the adaptive immune responses may, in certain circumstances, exacerbate the clinical course of a biofilm-associated infection and accelerate collateral tissue damage. The aim of the present study was to compare the *in vitro* response of human peripheral blood mononuclear cells (PBMC) to biofilms and planktonic cells of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, two bacterial species particularly relevant in patients with cystic fibrosis or undergoing endovascular catheterization, respectively.
Materials and Methods: PBMC isolated from healthy donors were co-cultured with 24 h-old biofilms or with exponentially growing cells of both species. Following 24 h of co-culture, the expression of early activation markers and the levels of cytokines in the culture supernatants were assessed by flow cytometry, while biofilm biomass and architecture were evaluated by crystal violet staining, CFU count, and confocal laser scanning microscopy (CLSM).
Results: In the case of *P. aeruginosa*, around 20% of PBMC were activated in response to both biofilms and planktonic cells. In contrast, in the case of *S. epidermidis*, the planktonic cells induced a statistically higher degree of activation than the biofilms (27% *versus* 16%; P <0.01). *P. aeruginosa*-biofilms stimulated pro-inflammatory (TNF-alfa, IL-1beta, IFN-gamma, IL-6) and anti-inflammatory (IL-10) cytokine-production at statistically higher levels than its planktonic counterpart, while an opposite trend was observed for *S. epidermidis*. CLSM data showed a thicker and more compact structure of the biofilm and a higher matrix-per-bacteria content in *S. epidermidis* than in *P. aeruginosa*. Live imaging of the interaction between PBMCs and biofilms supported these observations, demonstrating a greater number of cells penetrating the biofilm of *P. aeruginosa* compared to that of *S. epidermidis*.
Discussion and Conclusions: Collectively the results obtained highlighted marked differences in the host-cell response depending on the species and/or the mode of growth (biofilms *versus* planktonic cultures) and suggested different strategies adopted by *P. aeruginosa* and *S. epidermidis* to persist in the host.

126 - Long-term anti-bacterial immunity against systemic infection by Salmonella enterica serovar Typhimurium elicited by a GMMA-based vaccine

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Introduction. *Salmonella* Typhimurium (STm) represents the most prevalent cause of invasive non-typhoidal Salmonella (iNTS) disease, and currently no licensed vaccine is available.

Materials and Methods. In this work we characterized the long-term anti-bacterial immunity elicited by a STm vaccine based on Generalized Modules of Membrane Antigens (GMMA) delivering O:4,5 antigen, using a murine model of systemic infection.

Results. Subcutaneous immunization of mice with STmGMMA/Alhydrogel elicited rapid, high, and persistent antigen-specific serum IgG and IgM responses. The serum was bactericidal *in vitro*. O:4,5-specific IgG were also detected in fecal samples after immunization and positively correlated

with IgG observed in intestinal washes. Long-lived plasma cells and O:4,5-specific memory B cells

were detected in spleen and bone marrow. After systemic STm challenge, a significant reduction of

bacterial load in blood, spleen, and liver, as well as a reduction of circulating neutrophils and G-CSF glycoprotein was observed in STmGMMA/Alhydrogel immunized mice compared to untreated animals.

Discussion and Conclusions. Taken together, these data support the development of a GMMA-based vaccine for prevention of iNTS disease.

94 - Effectiveness of 3-O-methylfunicone against Bovine herpesvirus 1 infection

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Introduction - Bovine herpesvirus 1 (BoHV-1), a member of the alphaherpesvirus subfamily, is an important pathogen responsible for significant economic losses to the cattle industry and trade restriction. Due to its immunosuppressive properties, it can provoke infectious bovine rhinotracheitis, conjunctivitis, abortions or complicated polymicrobial infections. Indeed, BoHV-1 is a significant cofactor for bovine respiratory disease complex, the most important inflammatory disease in cattle, leading to pneumonia and occasionally to death. To date, non-toxic active drugs against BoHV-1 are not available yet, but fungal microorganisms represent a rich source of bioactive compounds with biological and antimicrobial activities. For example, some secondary metabolites produced by the species *Talaromyces pinophilus* such as 3-*O*-methylfunicone (3-OMF) showed significant antifungal and antiviral activities. In a recent screening, it has been observed that sulochrin and analogues, such as 3-OMF, reduce the infectivity of hepatitis C virus. Based on this evidence, this study aimed to evaluate the effectiveness of 3-OMF against BoHV-1 infection.

Materials and methods - 3-OMF was obtained by extraction and chromatographic purification of culture filtrate of *T. piniphilus* (strain LT6). MDBK cell line, *in vitro* bioscreen, cytomorphological and immunofluorescence analysis.

Results - Following BoHV-1 infection in MDBK cells, the non-toxic concentration of 5 □M 3-OMF significantly decreased cell death. Giemsa staining revealed in infected groups morphological features of cell death that were intensely reduced by the presence of 3-OMF. All these results were accompanied by a significant decrease in cytopathic effect in the presence of 3-OMF. In addition, 3-OMF strongly inhibited the expression of bICP0, the major regulatory protein in lytic cycle of BoHV-1.

Discussion and Conclusions - Overall, our preliminary results support the concept that 3-OMF may represent a potential antiviral agent in containing BoHV-1 infection. Further investigations are needed to clarify the mechanism by which 3-OMF may regulate BoHV-1 infection.

40 - Palmitoylethanolamide in the prevention of SARS CoV-2

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Introduction: The global SARS CoV-2 pandemic offered a new goal: finding effective drugs to fight or prevent this infection since no specific drugs have been identified yet.
Molecular docking analysis has identified Palmitoylethanolamide (PEA) as a potential ligand for Spike protein of SARS CoV-2 and for the cellular receptor ACE2. This binding could inhibit the interaction of the virus with ACE2 and prevent its entrance into cells.
PEA is an endogenous amide, largely present in various living organisms, its production occurs constitutively but is diminished after various pro-inflammatory stimuli.
Micronized PEA preparations are commonly used as analgesic, anti-inflammatory, and neuroprotective mediators.
This study aims to test PEA as inhibitor of SARS CoV-2 entry and characterize a possible antiviral role of this molecule.
Materials and Methods: Reference strain of SARS CoV-2 and the principal variants of concern (B.1.1.7, B.1.351, P.1 and B.1.617.2) obtained from clinical isolates were used for the infection of susceptible HuH-7 cells. Various experimental conditions, concentrations and time-points were assessed. Viral infections were assessed using qRT-PCR performed on supernatants and immunocytochemistry performed on infected cells, using high content confocal microscopy.
SARS CoV-2 Spike protein-pseudotyped GFP-lentiviral particles were incubated with different concentrations of PEA for 30 minutes and then used for HuH-7 transduction. Percentage of transduced cells were evaluated by Flow Cytometry and High Content Confocal Microscopy.
Statistical analyses were performed using One-way Anova. Data are expressed as mean $\pm$ SD in three biological and technical replicates.
Results: PEA at 1 and 10 μM reduces viral infection rate of SARS CoV-2 clinical strains. When PEA is administered at 100 μM increases viral spread. 1 and 10 μM PEA pre-incubation with Spike-pseudotyped lentiviral vectors caused a reduction of transduced cells by 37% and 18% respectively. PEA at 100 μM did not affect transduction levels.
Discussion and Conclusions: Early clinical trials with PEA suggested that the compound reduced the incidence of acute respiratory infections.
Our data strongly suggest an effect of PEA against SARS CoV-2 entry and/or replication when administered at low concentrations (1 and 10 μM). Higher concentrations of PEA show an opposite effect on cells, possibly due to unpredicted toxicity that may facilitate viral entry. The results are promising towards the use of this molecule to fend off SARS CoV-2 infection.

151 - INHIBITORY ACTIVITY OF OPHTHALMIC SOLUTIONS AGAINST HSV-1 AND SARS-COV-2

Gianluigi Franci - Francesco Petrillo - Annalisa Chianese - Maddalena De Bernardo - Marilena Galdiero - Michele Reibaldi - Teresio Avitabile - Giovanni Boccia - Nicola Rosa - Massimiliano Galdiero

Background. Viral infections represent a threat to public health. Herpes simplex virus type 1 (HSV-1) and Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are responsible for most eye infections. The transmission of the virus occurs either by direct or indirect contact, by means of air droplets, and can affect the oral and ocular areas. In order to prevent this specific infection, it is necessary to increase preventive measures and reduce viral spread. In this work we will focus on the attempt to reduce ocular infection and transmission, testing the antiviral activity against HSV-1 and SARS-CoV-2 of some already marketed eye drops (Iodim, Ozodrop, DROpsept and Septavis). **Materials and Methods**. HSV-1 (strain SC16) and SARS-CoV-2 (strain VR PV10734) were propagated on Vero cells, *Cercopithecus aethiops* kidney epithelial cells (ATCC CCL-81). To evaluate the mechanism of action of the compounds, several antiviral assays (co-treatment, virus pre-treatment, cell pre-treatment, and post-treatment) were performed. In each assay, different volumes of compounds (12.5, 25, 50, 100 µl) were analyzed in the presence of the virus (10^3 PFU/cell) for different stimulation times (15 sec, 30 sec, 1 min, 5 min, 10 min, 15 min, 30 min, 1 h, and 2 h). Furthermore, to confirm the data obtained by plaque assay, molecular tests were performed to quantify the expression of specific viral genes. **Results.** The data obtained indicate that only 2 of the 4 ophthalmic solutions (Iodim and Ozodrop) showed high inhibitory activity against HSV-1 at different times and volumes analyzed. While, against SARS-CoV-2, 3 of the 4 ophthalmic solutions (Iodim, Ozodrop, and DROpsept) showed significant antiviral activity under the same conditions tested. Furthermore, the data obtained *in vitro* were confirmed by molecular tests. The expression of the nucleocapsid (N) protein was inhibited by the action of the eye drops, while the expression of the spike (S) protein was reduced. **Conclusions**. Based on the data obtained, it is possible to identify these ophthalmic solutions as a preventive resource for eye infections caused by viruses (HSV-1 and SARS-CoV-2).

7 - Antimicrobial peptides HBD-2 and HBD-3 inhibit the intestinal biofilm of Pseudomonas aeruginosa and Staphylococcus aureus

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Introduction: intestinal microbiota is a very active microbial community where most of microbial colonisers are innocuous, beneficial and able to mutually interact with the host to play a role in homeostasis maintenance and in protecting the host from the environment; In addition to the microbiota, the cells of the intestinal epithelium represent the second barrier in the line of defense between the host and the environment; this is possible by the presence of tight junctions (TJs), which physically strengthen the barrier, and by the production of molecules, such as cytokines, chemokines and antimicrobial peptides (AMPs), able to activating the mechanisms of the innate immune system. The two main classes of AMPs are constituted by cathelicidins and defensins of which beta-defensin-2 and beta-defensin-3 (HBD-2 and HBD-3) are of particular importance. However, there are some circumstances in which an alteration of this eubiotic state occurs, with the triggering of dysbiosis. In this condition, the microbiota loses its protective power, leading to the onset of opportunistic infections. In this scenario, the emergence of multi-drug resistant biofilms from *Pseudomonas aeruginosa* and *Staphylococcus aureus* is very frequent. Due to the increased resistance to antibiotics and conventional treatment of bacteria in biofilms, finding substances or new therapeutic approaches to inhibit biofilm formation or trigger mature biofilm disassembly attracts considerable interest. Materials and Methods: *Cloning and transfection*: genes coding HBD-2 and HBD-3 were cloned and transfected into Caco-2 cells which were subcultured for 21 days to obtain their full differentiation. *biofilm evaluation*: overnight cultures of *P. aeruginosa* and *S. aureus* were diluted in cell culture supernatants of Caco-2, Caco-2/ HBD-2 and Caco-2/HBD-3 to a concentration of 10^7 CFUs/mL, placed into 96-well flat-bottomed sterile polystyrene microplates and incubated overnight at 37 °C. The evaluation of biofilm formation was carried out by crystal violet staining, by counting of viable sessile cells and by LIVE/DEAD staining with fluorescence microscopy. *Evaluation of expression of biofilm-associated genes:* The modulation of expression levels of biofilm-associated genes *lasI*, *lasR*, *pslA*, *ppyR*, (for *P. aeruginosa*) *icaAD* and *bap* (for *S. aureus*) was carried out by Real-Time PCR. Results: both HBD-2 and HBD-3 showed antibiofilm activity against *P. aeruginosa* and *S. aureus*; Discussion and Conclusions: The possibility of using endogenous antimicrobial peptides as new anti-biofilm therapy, in isolation or in combination with conventional antibiotics, can be an interesting prospect in the treatment of chronic and multi-drug resistant infections.

17 - Peroxisome proliferator-activated receptor-α is a novel antiviral target against SARS CoV-2

Michele Lai[1], Veronica La Rocca[1], Rachele Amato[1], Elena Iacono[1], Paola Lenzi[2], Francesco Fornai[2], Rossella Fonnesu[1],Giulia Lottini[1], Maria Sidoti[1], Pietro G. Spezia[1], Paola Quaranta[1], Giulia Freer[1], Daniele Piomelli[3*] and Mauro Pistello[1,4*]

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Introduction: current Covid-19 treatments aim to decrease the severe inflammatory responses and acute lung injury of patients. Since 2019, nine vaccines have been approved for use in humans. Two of the vaccines currently in use worldwide, BNT162b2 (manufactured by Pfizer) and mRNA-1273 (manufactured by Moderna), are based on lipid nanoparticle delivery of mRNA encoding a prefusion stabilized form of spike protein derived from SARS-CoV-2 isolated early in the epidemic from Wuhan, China. Both of these vaccines demonstrated >94% efficacy at preventing coronavirus disease 2019 (COVID-19). However, the recent emergence of novel circulating variants has raised significant concerns about geographic and temporal efficacy of these interventions. For this reason, a drug that blocks viral replication and limit the excessive inflammatory response to SARS CoV-2 is still an urgent need. Moreover, the discovery of new antiviral compounds might be beneficial for future spread of pandemic viruses.

After inflammation, Immune cells generate signals that counter inflammation and starts the recovery of tissues. Human cells possess a variety of signaling molecules to communicate with their surroundings and escalate the inflammatory response.
Lipid messengers involved in these reconstructive actions include the N-acylethanolamines (NAE), such as arachidonoylethanolamide (anandamide) and palmitoylethanolamide (PEA). PEA exerts its anti-inflammatory action through the peroxisome proliferator-activated receptor-α (PPAR-α) activation, a ligand-activated transcription factor. In this work we hypothesize that PPAR- α agonists might be beneficial to counteract SARS CoV-2 replication due to their ability to dismantle the lipid compartments that are fundamental for viral replication. Moreover, activating PPAR-α might maintain active autophagy that, acting as a second line of resistance, will eliminate the clusters of viral proteins.

Materials and methods: clinical strains of SARS CoV-2 were used to infect Huh-7 cells pre-treated with different concentrations of PPAR-α agonists. Viral infections were assessed using qRT-PCR performed on supernatants and immunocytochemistry performed on infected cells, using high-content confocal microscopy.

Results: here we demonstrate a novel antiviral effect of PPAR-α agonists trough pharmacological or genetic Knock-Out on SARS CoV-2 infected cells. Our findings revealed that SARS CoV-2 decrease viral replication and virion release by 5 times on PEA-activated cells. The same results were achieved using PPAR-α agonists at 1 μM. Both approaches sustain mitochondrial and peroxisomal β-oxidation.

Conclusions: this work highlights a novel antiviral target that can both reduce SARS CoV-2 replication and the iper-activation of the innate immune system.

73 - Role of endosomal Toll-Like Receptors in the etiopathogenesis of skin infections caused by Staphylococcus aureus

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Introduction: *Staphylococcus aureus* is an encapsulated Gram-positive bacterium that cause a wide variety of clinical diseases. Over the last 70 years, infections caused by this pathogen have become progressively more frequent, more severe and more difficult to treat due to the emergence of highly virulent and antibiotic resistant clones. Identification of the receptors involved in innate immune recognition of *Staphylococcus aureus* is essential to develop alternative strategies to treat infections caused by antibiotic resistant strains.

Materials and Methods: we examined the role of endosomal Toll-like receptors (TLRs), which sense the presence of prokaryotic-type nucleic acids, in anti-staphylococcal host defenses using different infection models involving genetically defective mice.

Results: Single deficiencies in TLR7, 9 or 13 resulted in mild or no decrease in host defenses. However, the simultaneous absence of TLR7, 9 and 13 resulted in markedly increased susceptibility to *S. aureus* infection, concomitantly with decreased production of pro-inflammatory chemokines and cytokines, neutrophil recruitment to infection sites and reduced production of reactive oxygen species. This phenotype was significantly more severe than that of mice lacking TLR2, which senses the presence of staphylococcal lipoproteins. Notably, the combined absence of TLR7, 9 and 13 resulted in complete abrogation of interleukin-12 p70 and interferon-beta responses to staphylococcal stimulation in macrophages.

Discussion and Conclusions: Our data highlight the presence of a highly integrated endosomal detection system, whereby TLR7, 9 and 13 cooperate in sensing the presence of staphylococcal nucleic acids. We demonstrate that the combined absence of these receptors cannot be compensated for by cell surface-associated TLRs, such as TLR2, or cytosolic receptors. These data may be useful to devise strategies aimed at stimulating innate immune receptors to treat *S. aureus* infections.

21 - In vitro analysis of SARS-CoV-2 infection in Cystic Fibrosis human bronchial epithelial cell line

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Introduction. The novel coronavirus disease 19 (COVID-19) caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) results in a pandemic in late 2019. Cystic fibrosis patients should be considered at higher risk of developing severe symptoms in case of COVID 19 illness since their reduced survival due to chronic pulmonary infections. Nonetheless, so far, several evidences reported a reduced spread of SARS-CoV-2 in CF subjects. The aim of this work was to investigate the peculiar characteristics of SARS-CoV-2 infection in CF patients using cell models with a genetically modulated CFTR expression.

Material and Methods. We have infected two different human bronchial epithelial cell lines (CFBE41o- and 16HBE14o-, with and without CFTR modification) with two different SARS-CoV-2 strains. Samples were collected before the infection and at 24, 48, and 72 hours post infection. RNA was extracted from both cell pellets and supernatants and SARS-CoV-2 target genes were amplified by both multiplex RT-PCR and Allplex 2019-nCoV assay kit (Seegene). To elucidate the role of ACE-2 receptor in this experimental condition, flow cytometry and western blot analysis were performed. A CFTR selective inhibitor has been used to shed the light on the involvement of CFTR in this infection mechanism.

Results. We reported a decrease of viral load in CF compared to WT cell lines, correlated with a lower expression of ACE-2 receptor in cell membrane of CF than control cells. Nonetheless, total ACE-2 protein expression did not show significant differences in CFTR-modulated cell line compared to WT, while in CFTR-deleted cell line a lower ACE-2 expression was appreciable in comparison with isogenic one. We have also blocked CFTR channel in WT cells inducing a significant drop of the viral load.

Discussion and Conclusion. CF cell lines showed a significant decrease of SARS-CoV-2 infection when compared with wild type cell lines. Although no difference was detected in ACE-2 total expression, ACE-2 protein membrane expression is slightly reduced on cell membrane of CF cell lines. Interestingly, the functional inhibition of CFTR channel elicited a significant decrease of SARS-CoV-2 replication. In conclusion, these data suggest that SARS-CoV-2 replication may be impaired by different mechanisms involving ACE-2 expression and CFTR functional modulation, by inducing an alteration of intracellular ionic balance and organelles pH levels, which could in turn affect cell receptor expression as well as virus replication and assembly.

43 - Increased competence of Nicotine treated pneumocytes for SARS-COV-2 infection

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1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic has a variable degree of severity according to underlying comorbidities and lifestyle. Many studies have reported an association between cigarette smoking and increased severity of COVID-19. The exact mechanism of action is nowadays largely unclear. We investigated whether angiotensin-converting enzyme 2 (ACE2) is overexpressed in human pneumocytes after exposure to nicotine, and if this leads in turn to increased SARS-CoV-2 replication and cytopathic effect.

2. Materials and Methods

Nicotine was assayed by *in vitro* experiments for its capacity to stimulate ACE2-expression in human pulmonary adenocarcinoma A549 epithelial cells. We exposed low ACE2-expressing A549 cells to nicotine and assessed ACE2 expression at different time points. Furtherly, we used the nicotine-exposed cells in plaque reduction neutralisation test (PRNT).

3. Results

Nicotine exposure induces rapid and long-lasting increases in gene and protein expression of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor ACE2, which in turn translates into increased competence for SARS-CoV-2 replication and cytopathic effect.

4. Discussion and Conclusions

These findings show that nicotine makes worse SARS-CoV-2 pulmonary infection and have implications for public health policies.

72 - Evaluation of antimicrobial activity of alcohol based hand sanitizers

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Introduction: Hands hygiene can be considered a crucial strategic key for reducing the colonization and incidence of infectious diseases in all populations. In case of absence of soap and water, the use of hand sanitizers are the most recommended as they are quick and easy to use and they are also not very aggressive for the skin. For this reason, alcohol based and non alcoholic hand sanitizers can be considered the most valid products presents on the market. The aim of this study was the evaluation of the antimicrobial activity of alcohol based hand sanitizers composed by different concentrations of three gelling agents: Carbopol 940, Hydroxypropylmethyl cellulose (HPMC), and Hydroxyethyl cellulose (HEC) against clinical and non-clinical bacterial strains. **Materials and Methods**: For the evaluation of antimicrobial activity a working culture for each organisms was prepared following the normative EN 1500:2013. A portion of 10 µl of working colture was added into 96 wells and 200 µl of alcohol based hand sanitizer was put in contact for 60s at room temperature. At the end of the incubation time the whole content of each well was seeded on TS agar and incubated overnight at 37°C and then viable cells count was evaluated. **Results:** The results showed that for the Gram negative the Carbopol 940 formulation drastically reduced the viability of strains tested with a reduction ≥ 7 Log CFU while HPMC formulation and HEC formulation reduce the viability of some strains of *E. coli ESBL* and *A. baumanni* to 6 - 5 Log CFU. For the Gram positive strains, the Carbopol 940 formulation reduced the viability of *S. aureus* strain ATCC and *E. hirae* ATCC with 8 Log CFU reduction; whereas, the HEC formulation reduced the viability of *S. aureus* MRSA strain 2 with a decrease of 7 Log CFU and the HPMC formulation *S. epidermidis* strain with a reduction of 8 Log CFU. Moreover, the hand sanitizer formulations tested were significantly active against *C. albicans* strain. **Discussion and Conclusions:** In conclusion, all tested solutions have a fair antimicrobial activity, especially Carbopol 940 based hand sanitizers which exhibit a more marked antimicrobial activity for all bacteria strains compared to hand sanitizers containing HEC and HPCM agents.

137 - CD169 expression on monocytes is involved in SARS-CoV-2 infection and is associated to clinical features of COVID-19

ANTONELLA MINUTOLO[1], VITA PETRONE[1§], MARIALAURA FANELLI[1§], CHRISTIAN MARACCHIONI, MARCO IANNETTA[2,3], MARTINA GIUDICE[1], INES AIT BELKACEM[4,5], MARTA ZORDAN[2,3], PIETRO VITALE[3], PAOLA SINIBALDI VALLEBONA[1,6], LOREDANA SARMATI[2,3], MASSIMO ANDREONI[2,3], FABRICE MALERGUE[5], GUIDO RASI[1], PAOLO DI FRANCESCO[1], EMANUELA BALESTRIERI[1], CLAUDIA MATTEUCCI[1], SANDRO GRELLI[1,7].

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Introduction: The COVID-19 is an acute infectious disease caused by the SARS-CoV-2 virus. To date, a standard therapeutic approach for COVID-19 patients (COV) has not been established and the identification of early biomarkers to predict disease progression is needed. Recently, was suggest that SARS-CoV-2 infects CD169 macrophages in the spleen and lymphonodes playing a central role in mediating SARS-CoV-2 translocation. Moreover, CD169 was strongly overexpressed in the blood of confirmed COV. To clarify whether CD169 was activated by SARS-CoV2 stimulation, Peripheral Blood Mononuclear Cells (PBMCs) from HDs were stimulated in vitro with SARS CoV-2 Spike protein for 24 hours and CD169 RMFI and mRNA expression were evaluated. Then, we analysed CD169 in blood cells of COV admitted to the hospital during the COVID-19 outbreak and correlated its expression with clinical characteristics. **Material and Methods**: The ratio of the Median Fluorescence Intensity (MFI) of CD169 between monocytes and lymphocytes (CD169 RMFI) was used to screening blood samples of Healthy Donors (HDs) and COV by flow cytometry, and its correlation with clinical signs, inflammatory markers, cytokines mRNA expression, and disease progression was evaluated. **Results**: *In vitro* stimulation of PBMCs from HDs with SARS-CoV-2 Spike protein induced a significant increase in CD169 RMFI in a dose-dependent manner with a significantly increase of IL-6 and IL-10 gene expression. CD169 RMFI was also highly expressed in the macrophages of COV but not in those of HDs, especially in untreated patients at sampling. In CD4+ T cells of untreated patients, CD169 RMFI inversely correlates with the expression of central memory (CD45RA- CCR7+) and effector memory (CD45RA- CCR7-) cells and directly correlated with exhaustion markers (CD57+ PD1+). In CD8+ T cells, its expression was associated with the decrease of naive (CD45RA+ CCR7+) and increase in EM (CD45RA- CCR7-) cells. Finally, CD169 RMFI positively correlated with the senescence marker CD57+. Moreover, the CD169 RMFI correlated with inflammatory markers, blood cytokine levels, and pneumonia severity in the untreated group of COV at sampling. Notably, in this group, CD169 reflects the respiratory outcome of patients during hospitalization. **Discussion and Conclusion**: our data highlighted the association between CD169 expression and clinical status, inflammatory markers, and respiratory outcome and considering the immunological role of CD169 and its involvement during the infection and the progression of COVID-19, it could be considered as an early biomarker of disease progression.

140 - CD169 expression on monocytes is involved in SARS-CoV-2 infection and is associated to clinical features of COVID-19

ANTONELLA MINUTOLO[1], VITA PETRONE[1§], MARIALAURA FANELLI[1§], CHRISTIAN MARACCHIONI, MARCO IANNETTA[2,3], MARTINA GIUDICE[1], INES AIT BELKACEM[4,5], MARTA ZORDAN[2,3], PIETRO VITALE[3], PAOLA SINIBALDI VALLEBONA[1,6], LOREDANA SARMATI[2,3], MASSIMO ANDREONI[2,3], FABRICE MALERGUE[5], GUIDO RASI[1], PAOLO DI FRANCESCO[1], EMANUELA BALESTRIERI[1], CLAUDIA MATTEUCCI[1], SANDRO GRELLI[1,7].

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98 - Can bacterial and viral endosymbionts influence the susceptibility of Trichomonas vaginalis to metronidazole?

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Introduction: *Trichomonas vaginalis* is a flagellated protozoan pathogen of the human urogenital tract, able to establish endosymbiotic association with two *Mycoplasma* species (*Mycoplasma hominis* and Candidatus *Mycoplasma girerdii*) and with dsRNA viruses (TVV). Metronidazole and nitroimidazole derivatives remain the only approved treatment of protozoan infection. The first case of nitroimidazole resistance in women was reported in 1962, but recent studies estimated that the prevalence of drug resistance varies from 2 to almost 20% upon geographic distribution. *Mycoplasma* and TVV presence in *T.vaginalis* isolates was recently associated with metronidazole resistance of clinical strains.

In the current work, we assessed the prevalence of *M.hominis*, '*Ca*.M.girerdii' and TVVs in *T. vaginalis* clinical isolates in Italy and Vietnam, evaluating the association between the presence of symbionts and *in vitro* metronidazole susceptibility in protist. In addition, RNA-Seq analysis has led to study the effects of *Mycoplasma* endosymbionts on expression of a group of protozoan genes associated with drug susceptibility.

Materials and methods: Sixty-five *T.vaginalis* isolated were investigated for the presence of endosymbionts, and metronidazole susceptibility was determined. In addition, we set up an experimental model based on single and double *Mycoplasma in vitro* infection of *Mycoplasma*-free isogenic *T.vaginalis*, in order to investigate the expression profiles of genes associated with metronidazole, by RNA-Seq techniques.

Results: The prevalence rate of *Mycoplasma*-positive Vietnamese isolates was 38% compared with 88% of Italian *T.vaginalis* strains. Among Vietnamese trichomonad strains, only 9% showed TVV presence. Antimicrobial assays showed that all isolates tested were drug sensitive, although *Mycoplasma* infected strains showed a lower sensibility to metronidazole compared with *Mycoplasma*-free isolates. In addition, RNA-Seq experiments with isogenic experimentally infected strains, have shown a slight significant decrease in pyruvate:ferredoxin oxidoreductase (PFOR) genes expression in *Mycoplasma*-infected *T.vaginalis*, compared with mycoplasma-free controls.

Discussion and Conclusions: Our data confirmed both the high variability of association rate between symbionts and *T.vaginalis* and the capability of protist to establish endosymbiotic relationship with *M.hominis*, '*Ca*.M.girerdii' and TVVs. We also demonstrated that bacterial endosymbionts can influence the metronidazole sensibility in mycoplasma-infected *T.vaginalis* strains.

6 - Caspase-8 regulates the switching autophagic-like/apoptotic response to herpes simplex 1 infection in apoptosis prone cells

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Introduction. Not all cell types undergo the same fate in terms of cytopathic effect in response to herpes simplex viruses (HSV) infection. In fact, cells which highly sustain virus replication, such as epithelial cells, invariably undergo lytic necrosis following HSV infection, while cells which do not optimally sustain virus replication, such as human monocytic cells, dendritic cells or lymphocytes, or cells from mice, such as mouse embryonic fibroblasts (MEFs), exhibit apoptotic regulated cell death (RCD) as an exclusive cytopathic effect. However, complex mechanisms controlling RCD during infection by HSV have not fully elucidated. Thus, the present study was focused on the role of Caspase-8 in HSV-induced RCD. In fact, data from other authors suggested that this caspase was implicated in RCD caused by HSV infection, while results of our studies excluded a role for death receptors in the same process. Materials and Methods. Human monocytic U937 cells and their stable transfectants dominant-negative for NF-kB, mouse embryonic fibroblasts (MEFs), wt or knock-out/knock-down for caspase-8, FADD, Bax/Bak, or HEp-2 human epithelial cells were used for HSV-1 experimental infection. Vero cells were used for the "F" strain of HSV-1 production. Caspase-3 and Caspase-8 activities were measured by fluorogenic assays or by Western blot analysis. The later technique was utilized also for Beclin-1 or LC3-I/LC3-II analysis. Immunofluorescence by optical or confocal microscopy and transmission electron microscopy were used to detect HSV-1 infection and RCD- or autophagic-like-related cell features. Cell viability was detected by classical assays. Results and Conclusions. HSV-1 activated Caspase-8 in apoptosis-prone infected cells. HSV-1-induced cell death was hindered in C8-KO-MEFs and in HSV-1 infected MEFs Caspase-8 functionality was required not only for apoptotic RCD but also for virus release. FADD adaptor protein was not implicated in Caspase-8-driven RCD in response to HSV-1 infection and recruitment of Caspase-8 in MEFs occurred downstream of the mitochondrial signalling. Beclin-1 cleavage was clearly detected in wt MEFs, but not in caspase-8 KO MEFs infected by HSV-1. Moreover, LC3-I/LC3-II conversion in response to HSV-1 infection remarkably occurred only in MEFs in which caspase-8 cleavage was not achieved. Actually, experiments carried out by confocal and electron microscopy clearly showed that in caspase-8 KO cells infection by HSV-1 stimulated a strong perinuclear autophagic-like vesicular response, with entrapped virions in cellular endosomes. Finally, pharmacological inhibition of PI3-kinase restored the ability of HSV-1 to induce apoptotic RCD in C8-KO-MEFs. In this context, Caspase-8 functionality in HSV-1-induced RCD seems related to a negative inhibition of the autophagic-like response as a necessary mechanisms for completion of apoptotic RCD. All together our results provide support for a non-canonical role of caspase-8 in HSV-1 infection as the possible regulator for the switching of autophagic-like and apoptotic response in cells prone to apoptosis following infection.

122 - Expression of the pathogenic HERV-W envelope in swab samples and in T lymphocytes in association with the respiratory outcome of COVID-19 patients

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Introduction: The identification of early biomarkers for predicting Coronavirus Disease 2019 (COVID-19) progression and of new therapeutic intervention for patient management are needed, considering that no standard therapeutic approach has been established yet. As recent findings that the Human Endogenous Retrovirus-W Envelope (HERV-W ENV) is activated in response to infectious agents and leads to various immune-pathological effects, the present study aimed to evaluated HERVs involvement during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. **Materials and Methods:** Residuals of naso-oropharyngeal swabs samples were collected at "Tor Vergata" University Hospital of Rome and the expression of HERV-K and HERV-W ENV, pro-inflammatory mediators, and also SARS-CoV-2 infection-related genes Angiotensin-converting enzyme 2 receptor (ACE2) and Nucleocapsid gene (N) have been analyzed by RT-Real time PCR. Moreover, HERV-W ENV expression in blood samples of hospitalized COVID-19 patients was analysed by flow cytometry and quantitative RT-PCR, and correlated with clinical signs, inflammatory markers, cytokine expression, and disease progression. **Results:** A significant increase of the HERV-K and HERV-W ENV activity in parallel with the higher expression of pro-inflammatory mediators and ACE-2 and N gene has been observed in SARS-CoV-2 positive compared to negative swab samples and higher levels have been found in hospitalized patients. Moreover, a positive correlation between HERV-W ENV and IL-6, IL-10, TNF-alpha expression has been found. HERV-W ENV has been found expressed, both as mRNA and protein, in blood samples from COVID-19 but not in HDs. Lymphocytes displayed the highest values among all leukocytes, and $CD3^+$ T cells showed the highest percentage of HERV-W ENV positive cells and correlated with the T cell differentiation, exhaustion, and senescence markers. Moreover, the percentage of HERV-W ENV-positive $CD4^+$ T cells significantly correlated with coagulopathy and biochemical parameters associated with COVID-19 severity. Interestingly, a significant increase in the percentage of HERV-W ENV-positive lymphocytes across groups with different pulmonary involvement was observed. Notably, HERV-W ENV expression in swabs and in blood samples reflects the respiratory outcome of patients during hospitalization. **Discussion and conclusion:** The data suggest HERV-W ENV as potential early biomarkers of the disease severity and contributing factor in the development and progression of COVID-19 and candidate it as a new potential therapeutic target.

127 - Interplay between persistent uropathogenic Escherichia coli and bladder epithelial cells

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Introduction Uropathogenic *Escherichia coli* (UPEC) is the major causative agent of urinary tract infections (UTIs). UPEC initially colonizes the human host adhering to the bladder epithelium. Once internalized in bladder epithelial cells, the bacteria can persist for a long time in a quiescent state, and this process enables the persistent bacteria, to organize into an intracellular bacterial community (IBC) to escape host cell elimination and actively resurge from the reservoirs. When chemotherapy fails bacteria might ascend the ureters and reach the kidneys, this may result in irreversible kidney failure and/or septicemia. In men, prostate infection frequently accompanies cystitis, supporting the case for bacterial prostatitis to be classified as a UTI. In this study UPEC strains, isolated from a patient with recurrent UTI, were phenotypically and genotipically characterized and assayed for invasion ability in bladder cell model.

Material and Methods UPEC strains were collected over a 10 years span from a patient with recurrent UTI (cystitis pyelonephritis) and EC73 was an invasive and persistent strain in prostate cells. CFT073 and *E. coli* K-12 MG1655 were used as reference strains. The genetic relationships among UPEC were determined by RAPD analysis. The isolates were characterized for biochemical and antibiotic resistant profiles and hemolytic activity. Biofilm formation ability was established by crystal violet staining while swimming motility was determined by measuring the diameters of the swimming zone in soft agar plates. The invasive and intracellular survival abilities of UPEC strains in bladder T-24 cell monolayers were assessed by the gentamicin protection assay.

Results RAPD analysis revealed that the isolates were divided into three clonal groups. The biochemical and antibiotic resistant spectra of the UPEC strains correlated with the RAPD profile. Interestingly, some strains were lactose negative, multidrug resistant and weak biofilm producers, but they were excluded for invasive assays because of gentamycin resistance. Some UPEC *E. coli* isolates, as long as reference CFT073 and EC73 strains, were able to entry and persist into T-24 bladder cells. The moderate biofilm producer was not invasive in bladder cells and similarly to EC73 (strong biofilm producer and persistent strain) was less able to move in soft agar and more resistant to high temperature compared to other strains.

Discussion and Conclusions These preliminary results confirm that different factors could play an important role in persistence of UPEC including metabolism, antibiotic resistance, flagella biosynthesis, biofilm formation and bacterial invasion ability. Whole genome sequencing of UPEC strains to deepen their genomic and functional features is in progress.

13 - Modulation of *Candida albicans* biofilm formation by cell-free supernatant of *Lactobacillus iners*

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Introduction

Candida albicans is a commensal yeast commonly found in vaginal flora. The shift to opportunistic pathogen is driven by intrinsic and extrinsic factors that are still under debate. Several studies have reported vaginal dysbiosis during vulvovaginal candidiasis (VVC), with *L. crispatus* progressively replaced by *L. iners. To date the role of L. iners in the pathogenesis of VVC was scarcely investigated.* The aim of this study was to evaluate the effect of cell-free supernatant (CFS) of *L. iners* in the modulation of *C. albicans* biofilm formation, which is present in nearly 90% of VVC cases.

Materials and Methods

C. albicans clinical isolates (n=8) obtained from women affected by vulvovaginal candidiasis attending the microbiological diagnostic service of the University Hospital Santa Maria della Misericordia, Perugia were screened for biofilm formation ability after 24, 48 and 72 h of incubation. Biofilm biomass was quantified after crystal violet staining. Selected strong and moderate biofilm-forming strains were then treated during biofilm formation with CFS of a 24-h culture of *L. iners* ATCC 55195 and evaluated through CV staining and XTT assay. 24-h-old biofilms were also examined by light microscopy for hyphal formation.

Results

All the clinical isolates were able to form a mature biofilm within 24 h of incubation. *C. albicans* strains classification showed that the majority (n=5) was moderate biofilm producers and the remaining three were strong biofilm producers. Strong biofilm-forming strains did not show modulation in presence of *L. iners* CFS in term of biofilm biomass and metabolic activity. Conversely, moderate biofilm-forming strains showed a statistically significant increase in biofilm biomass production and metabolic activity. In addition microscopy analysis show that *L. iners* CFS is able to induce hyphal morphogenesis of the strains classified as moderate producers of biofilm.

Discussion and Conclusions

The pathogenesis of VVC still remains controversial. These results are the first that show the effect of *L. iners* CFS on the increase of an important virulence factor of *C. albicans* like biofilm formation. These new data give more and more importance to the study of the microbiota and the complex system of interactions that take place at the vaginal level regulating the balance between health and disease. In particular, these results suggest an important role of *L. iners* in the pathogenesis of VVC.

39 - Splenic macrophages drive bacteraemia during pneumococcal pneumonia

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Abstract

Introduction: Severe community-acquired pneumococcal pneumonia is commonly associated with bacteraemia. Although it is assumed that the bacteraemia solely derives from pneumococci entering the blood from the lungs it is unknown if other organs are important in the pathogenesis of bacteraemia. Using three models, we tested the relevance of the spleen in pneumonia-associated bacteraemia.
Materials and Methods: We used human spleens perfused *ex vivo* to explore permissiveness to bacterial replication, a non-human primate model to check for splenic involvement during pneumonia and a mouse pneumonia-bacteraemia model to demonstrate that splenic involvement correlates with invasive disease.
Results: Here we present evidence that the spleen is the reservoir of bacteraemia during pneumonia. We found that in the human spleen infected with pneumococci, clusters with increasing number of bacteria were detectable within macrophages. These clusters also were detected in non-human primates. When intranasally infected mice were treated with a non-therapeutic dose of azithromycin, which had no effect on pneumonia but concentrated inside splenic macrophages, bacteria were absent from the spleen and blood and importantly mice had no signs of disease.
Discussion and Conclusions: We conclude that the bacterial load in the spleen, and not lung, correlates with the occurrence of bacteraemia. This supports the hypothesis that the spleen, and not the lungs, is the major source of bacteria during systemic infection associated with pneumococcal pneumonia; a finding that provides a mechanistic basis for using combination therapies including macrolides in the treatment of severe community-acquired pneumococcal pneumonia.

80 - Transglutaminase 2 inhibitors as a future intervention against nontuberculous mycobacteria

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Background: Diseases caused by Non Tuberculous Mycobacteria (NTM) are on the rise worldwide, including immunocompetent adults. The current treatment against NTM infections is based on the combined use of antibiotics that are administered for 6-12 months. Despite these aggressive regimens, success rate barely exceed 50% due to the intrinsic and acquired NTM drug resistance. Host-directed therapies (HDTs) are emerging as a promising area of research and are opening new avenues in the treatment of mycobacterial infection. We recently demonstrated that genetic or pharmacological inhibition of transglutaminase 2 (TG2), a multifunctional ubiquitous enzyme that catalyses post-translational modifications of proteins in eukaryotic cells, results in the reduction of Mycobacterium tuberculosis replication in murine and human primary macrophages. In this study we aim to assess the anti-mycobacterial activity of the TG2 inhibitors cystamine and cysteamine against a panel of NTM.

Material and Methods: To test the potential activity of TG2-inhibitors, human monocyte-derived macrophages (THP-1) were infected with clinical isolates of NTM (*Mycobacterium avium (MAV)*, *Mycobacterium abscessus (Mabs)*, *Mycobacterium intracellulare (MAI))* and then treated with antibiotics currently used in the standard therapy or with cystamine or cysteamine. We also evaluate cysteamine and cystamine activity in the human *ex vivo* model of granuloma-like structures (GLS).

Result: Intracellular CFUs were determined at day 2 and a significant reduction was observed following treatment with these inhibitors. Interestingly, when THP-1 cells were infected with the two colony variants of *Mabs* (Smooth and Rough), the treatment with cystamine and cysteamine turns out to have a superior antimicrobial response compared with the drug streptomycin and especially against the most virulent R variant of *Mabs*. The study in the GLS model further confirmed the ability of these drugs to restrict NTM replication (reduction of 1.2 log CFU for Mabs; ≈ 2 log CFU for MAV and 1.3 for MAI) and to reduce the size of GLS.

Conclusion: The antimicrobial activity of the TG2-inhibitors synergized with a standard drug as amikacin in human monocyte-derived macrophages and in the GLSs model. Overall, the results of this study support the potential usefulness of the TG2-inhibitors cysteamine and cystamine as HDTs in NTM disease.

37 - EDTA and Taurolidine affect Pseudomonas aeruginosa virulence in vitro: impairment of secretory profile and biofilm production onto peritoneal dialysis catheters

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Introduction: peritoneal catheter-associated biofilm infection is reported to be the main cause of refractory peritonitis in peritoneal dialysis patients. The application of antimicrobial lock therapy, based on results on central venous catheters, may be a promising option also for treatment of biofilm-harboring peritoneal catheters. In this study, we investigated the effects of two lock solutions, EDTA and Taurolidine, on an "*in-vitro*" model of *Pseudomonas aeruginosa* biofilm-related peritoneal catheter infection.

Materials and Methods: silicon peritoneal catheters were incubated for 24 h with a bioluminescent strain of *P. aeruginosa*. After washing, serial concentrations of Taurolidine (0.5, 0.25 and 0.125 %) and EDTA (2.5, 0.75 and 0.25 %), either alone or in combination, were applied for 24 h, once or twice, onto the contaminated catheters and then *P. aeruginosa* viability/persistence was evaluated in real time up to 120 h, by a Fluoroskan reader. Moreover, on selected supernatants from biofilm treated or not with EDTA and/or Taurolidine, High-Performance Liquid Chromatography-Mass (HPLC) analysis was performed to measure phenazine and pyocianine production.

Results: Taurolidine alone or in combination with EDTA caused a significant decrease of bacterial load and biofilm persistence onto the contaminated catheters. The lock solution treatment did not lead to the sterilization of the devices; yet, it resulted in a substantial destructuration of the peritoneal catheter-associated *P. aeruginosa* biofilm. Moreover, HPLC analysis showed that the treatment of biofilm-harboring catheters with EDTA and Taurolidine deeply affected the secretion of some key virulence-related molecules by *P. aeruginosa*, such as phenazines and pyocianines.

Discussion and conclusions: EDTA and Taurolidine affect the formation and persistence of *P. aeruginosa* biofilm onto peritoneal catheters; moreover, also the secretion of *P. aeruginosa* virulence factors is profoundly compromised. Future studies are needed to establish whether such lock solutions can be used to render peritoneal catheter-related infections more susceptible to antibiotic treatment, thus avoiding/reducing the onset of the antibiotic resistance phenomena.

146 - In vitro SARS-CoV-2 infection of microvascular endothelial cells: effect on pro-inflammatory cytokines and chemokynes release

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Introduction: In the novel pandemic coronavirus disease 2019 (COVID-19), caused by the infection via angiotensin-converting enzyme 2 (ACE2) receptors of the Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2), cytokine storm represents one the major causes of acute respiratory distress syndrome and mortality. High levels of pro-inflammatory cytokines lead to endothelial activation and dysfunction which contribute to disease severity by promoting a pro-coagulative state, thrombotic events and microvasculature injuries. Despite these evidences, the direct effects of SARS-CoV-2 infection on endothelium remain unclear. Thus, the final aim was to investigate Human Microvascular Endothelial Cells (HMEC-1) infection by SARS-CoV-2 and its effects on pro-inflammatory cytokines and chemokines release.

Materials ad Methods: Western Blotting was performed to state if HMEC-1 express ACE2 receptor. SARS-CoV-2/human/ITA/Milan-UNIMI-1/2020 infection of HMEC-1 was assessed by specific one-step Reverse Transcriptase Quantitative Real Time Polymerase Chain Reaction (RT-qPCR) on viral RNA extracted from infected cells and supernatants until 7 days post-infection (PI), while virions localization was observed by Transmission Electron Microscopy (TEM). Interleukin-6 (IL-6), Tumor Necrosis Factor alpha (TNFalpha), C-X-C motif Chemokine Ligand 8 (CXCL8) and Interferon alpha family (IFNalpha) levels in supernatants from infected or uninfected HMEC-1 were evaluated by Enzyme-Linked ImmunoSorbet Assay (ELISA).

Results: Expression of ACE2 receptor on HMEC-1 was confirmed. SARS-CoV-2 infection course was evaluated by RT-qPCR showing a maintenance of the viral load from 24h PI until 7 days PI, with $1.66*10^5$ copies/µg in cellular RNA. Viral load remained unaltered also in supernatants until 7 days PI. This finding was corroborated by TEM analysis showing virions localized in cytoplasm.

Regarding cytokines and chemokines secretion, only IL-6 levels were significantly higher after 24h, 48h and 72h PI in supernatants from infected cells compared to uninfected HMEC-1 ($p<0.001$), while CXCL8 was significantly lower at 24h PI ($p<0.001$), with non-significant but lower levels also in infected HMEC-1 until 7 days PI. TNFalpha and IFNalpha were not detected either in uninfected and infected HMEC-1.

Discussion and Conclusions: These data indicate that microvascular endothelial cells can be susceptible of infection. Infection directly contribute to the increased levels of IL-6, the main mediator and biomarker of severe disease.

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

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Introduction: the transcriptional activity, protein and viral particle production of human endogenous retrovirus K (HERV-K) have been demonstrated in tissue, patient serum and cell lines isolated from different types of tumors, such as ovarian, breast, prostate, teratocarcinoma, lymphomas, leukemias, sarcomas and melanoma. The mechanisms underlying HERV-K oncogenic activity could depend on the expression of oncogenic viral proteins, on the induced immune escape mechanisms, on the regulation of gene expression mediated by the long terminal repeat sequences or by the ability of retro-transposition determining genomic instability and alteration of the expression of neighboring genes. In the field of onco-hematology some studies have identified alterations of HERVs messengers and proteins expression in human lymphoid leukemic cells and the presence of circulating antibodies to HERV-K. On these bases, we evaluated the potential use of distinct HERVs families as biomarkers of disease and prognosis of chronic lymphocytic leukemia (CLL). **Materials and methods:** Peripheral blood mononuclear cells (PBMCs) were collected from peripheral venous blood from 75 patients with CLL diagnosis and 59 healthy donors (HDs) recruited. CLL patients have been divided into three groups: 28 naïve/untreated, 18 undergoing chemotherapy, and 29 treated with biological drugs. In PBMCs, the transcriptional activity of ENV gene of HERV-K (HML-2), HERV-H, HERV-W and pathogenic HERV-W as well as of the embryonic genes OCT4 and KLF4 and stemness marker CD133, were analyzed by RT-Real Time PCR. Also, we characterize the B lymphocyte subpopulations ($CD19^{+}CD5^{+}$) and analyzed the percentage of HERV-K ENV positive cells by flow cytometry analysis. **Results**: The molecular analysis showed a significant higher expression of all genes analyzed in the patients compared to HDs. Moreover, a positive correlation with HERVs expression and embryonic genes in patients was demonstrated. Of note, we can discriminate the two distinct populations of HDs and patients based on the HERVs expression. We found significant differences in HERV-K, HERV-H, HERV-W, OCT4, and KLF4 expression between untreated patients and those treated with chemotherapeutic or biological drugs patients. Finally, we have preliminary data about higher level of ENV HERV-K protein in $CD19^{+}CD5^{+}$ cell in CLL patients than HDs. **Discussion and conclusion:** the results suggest HERVs expression of as distinctive markers of CLL and their involvement in etiopathogenesis of the disease. The ongoing study could provide an indication of the role of HERVs as markers associated with genetic instability and as prognostic factors, in order to identify subgroups of CLL patients who could benefit from targeted therapeutic approaches.

24 - KI and WU polyomavirus in oropharyngeal swabs of SARS-CoV-2 infected patients.

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Introduction: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), etiological agent of coronavirus disease 2019 (COVID-19) has been declared a global pandemic in March 2020 by the World Health Organization (WHO). Our goal was to determine whether co-infections with respiratory polyomaviruses, such as Karolinska Institutet polyomavirus (KIPyV) and Washington University polyomavirus (WUPyV) occur in SARS-CoV-2 infected patients. **Materials and Methods:** Oropharyngeal swabs from 150 individuals, 112 symptomatic COVID-19 patients and 38 healthcare workers not infected by SARSCoV-2, were collected from March 2020 through May 2020 and tested for KIPyV and WUPyV DNA presence. **Results:** Of the 112 SARS-CoV-2 positive patients, 27 (24.1%) were co-infected with KIPyV, 5 (4.5%) were positive for WUPyV, and 3 (2.7%) were infected simultaneously by KIPyV and WUPyV. Neither KIPyV nor WUPyV DNA was detected in samples of healthcare workers. Significant correlations were found in patients co-infected with SARS-CoV-2 and KIPyV ($p < 0.05$) and between SARS-CoV-2 cycle threshold values and KIPyV, WUPyV and KIPyV and WUPyV concurrently detected ($p < 0.05$). These results suggest that KIPyV and WUPyV may behave as opportunistic respiratory pathogens. **Discussion and Conclusions:** Additional investigations are needed to understand the epidemiology and the prevalence of respiratory polyomavirus in COVID-19 patients and whether KIPyV and WUPyV could potentially drive viral interference or influence disease outcomes by upregulating SARS-CoV-2 replicative potential.

131 - Gut microbiota characterization and expression of genes related to the serotonin (5-HT) pathway in paediatric patients with Chronic Intestinal Pseudo-Obstruction (CIPO).

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Introduction. Chronic intestinal pseudo-obstruction (CIPO) is a form of gastrointestinal dysmotility in absence of limiting or occluding lesions of the intestinal lumen. Serotonin (5-HT) release, a local mediator and neurotransmitter, is linked to intestinal peristaltic and secretory reflexes. The intestinal microbiota and the enteric nervous system interact through the release of serotonin, and the subsequent activation of its 5-HT4 receptor. Recent studies indicate that the microbiota is able to modulate the secretion of serotonin synthesized by enterochromaffin cells. Lili Gu reports that nine CIPO patients undergoing fecal microbiota transplantation showed significant improvements in bowel obstruction scores and symptoms of swelling and pain, and in the control of bacterial overgrowth (often associated with CIPO patients). All of this indicate that microbiota composition could play a role in the pathogenesis of CIPO disease. The interplay among ENS/5-HT and dysbiosis in CIPO remains largely unclear. The present research aim is to study alterations of gut microbiota and the expression regulation of genes related to the 5-HT pathway, i.e., serotonin synthesis (*TPH1*) and reuptake (*SLC6A4*), in the same biopsy samples. **Methods.** from 7 pediatric CIPO patients and 7 age-/sex-matched healthy controls we: (*i*) Assess mucosal-associated microbiota (MAM) from biopsies samples by next generation sequences (NGS) of the V3-V4 region of the bacterial RNA 16s, in a Miseq platform; (*ii*) Establish the expression of *TPH1* and *SLC6A4* genes by qPCR after total RNA extraction from the same tissue samples, *iii*) Perform analyses to highlight correlations between MMA and the expression of genes linked to the production and transport of 5-HT (*TPH1* and *SERT*), and clinical parameters of CIPO patients. **Results.** Preliminary results obtained indicate that CIPO MAM is changed in its composition and biodiversity, respect to controls. At species level, bacteria known as probiotics, resulted significantly higher in controls (*Faecalibacterium prausnitzii* and *Bifidobacterium bifidum* and *Akkermansia muciniphila*), while potentially pathogens (*Clostridium difficile* and *Pseudomonas veronii*) relatively more abundant in CIPO patients**. Discussion conclusion.** We evaluated if abnormalities in serotonergic signaling, that could be triggered/correlated by alteration of gut microbiota, could affect intestinal motility in pediatric CIPO patients. Preliminary results obtained showed, for the first time in CIPO patients, a MAM different in its composition and biodiversity, respect to controls. Studies on microbiota composition, especially the MAM, in patients with constipation are rare, and therefore very useful to better understand microbiota involvement in intestinal dysmotility.

22 - Characterization of human CMV infection in kidney epithelial cells reveals senescence-mediated mechanisms of disease

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INTRODUCTION. Human cytomegalovirus (CMV) is the major infectious cause of birth defects and an important opportunistic pathogen, although lifelong infection persists asymptomatically in most individuals. Diseases associated with CMV infection affect immunocompromised populations, especially transplant recipients and AIDS patients. In kidney transplant recipients, CMV infection/reactivation is associated with allograft rejection and organ-specific allograft injury. CMV pathogenesis has been broadly associated with "indirect effects," a term that indicates the large number of immunomodulatory functions encoded by CMV that lead to immunosuppressive and immunostimulatory phenotypes underlying CMV-mediated diseases. On the other hand, despite clear correlations between CMV, lytic infection and kidney disease development, the underlying pathogenetic mechanisms remain elusive in the context of the targeted organ. Cellular senescence is a physiological phenomenon but also a response to stress stimuli such as DNA damage, aging, and infections. Perhaps the most well documented therapy-induced acquisition of renal senescence is transplantation. Whether CMV could contribute to increase senescence in the transplant kidney with subsequent pathological outcomes has never been addressed. Our group previously reported that CMV infection in human fibroblasts triggers cell senescence which involves expression of the immediate early gene IE2. Here, we show that renal tubular epithelial cells undergo cellular senescence in order to support complete viral replication upon CMV infection.
MATERIAL AND METHODS. Primary kidney proximal tubular epithelial cells (RPTEC) and the kidney adenocarcinoma 786-O cell line were infected at MOI 1 with CMV 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 RPTEC *in vitro* and in tissues from a full-blown HCMV infection that occurred in a pregnant woman who experienced preterm delivery.
RESULTS. We experimentally demonstrated that RPTEC represent a valuable model of renal CMV-infection *in vitro*. Moreover, the transcriptional profile analysis provided evidence that inflammatory response and allograft rejection signatures are induced in CMV-infected RPTEC. Finally, we showed that CMV infection induces a senescent phenotype both *in vitro* and *in vivo*.

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

90 - Reverse genetics approaches to the study of Parvovirus B19

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Introduction

Parvovirus B19 (B19V) is a small human pathogenic virus with no specific antiviral strategies developed so far. Despite recent studies lead to the discovery of molecules able to counteract viral replication, approved treatments are still unspecific and symptomatic.

NS1, the main protein involved in B19V replication, is encoded in the left-hand region of the genome, while the capsid proteins (VPs) are encoded in the right-hand half.

With the goal of deepening the knowledge about NS1 and, in the meantime, of obtaining a proof of concept of the possibility to manipulate B19V genome, B19V genomic clones able to replicate were modified to split the expression of NS1 and VPs on different genomic units.

Materials and Methods

B19V genomic clones were modified via recombinant PCR or by substitution of a synthetic genomic fragment. Defective clones obtained through these mutageneses underwent a functional analysis by nucleofecting UT7/EpoS1 cells with single clones or a mixture of NS-only and VP-only encoding clones, then analyzing these cells by immunofluorescence and RT-qPCR.

Results

Three families of modified genomic clones were generated: two minigenomes (pAs1 and NS Spliced), able to express NS and VPs respectively and lacking the region encoding for the counterpart, and one NSKO genome where the starting codon of NS gene was silenced.

The expression levels of NS obtained from pAs1 minigenomes are comparable or higher than the ones from unmodified control clones. NS Spliced minigenomes showed a very low yield of VPs expression, such as NSKO genome, which can be partially recovered in the presence of NS protein.

Discussion and Conclusions

This study showed that a manipulation of B19V genome is possible, despite its small dimensions, and can lead to a functional complementation of different genomic regions. These clones could be used to better characterize the NS protein and its cellular partners looking for antivirals, as well as they could be modified for a transgene expression in UT7-EpoS1 cells or Erythroid Progenitor Cells (EPCs).

121 - Significant in vitro anti-HIV activity of a rare biscoumarin extracted from a Cameroonian Hypericum species

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Abstract:

Introduction: Natural molecules represent an essential source of innovative solutions for the prevention and therapy of HIV/AIDS. In recent years, members of the *Hypericum* genus have attracted great interest due to their interesting biological value. During the collection of Cameroonian *Hypericum* species for bioactive compound discovery, we heard from the local population that the plants are traditionally used against mental disorders by AIDS patients. We evaluated their anti-HIV properties to determine whether *Hypericum* species might hint toward a new anti-HIV lead compound from a medicinal plant.
Materials and Methods: The $CHCl_3$ extract obtained from *Hypericum roeperianum* and isolated biscoumarin were evaluated in *cell-based* assay for cytotoxicity, and anti-HIV-1 activity. Furthermore, the safety profile of biscoumarin has been assessed by Transepithelial Electrical Resistance (TEER) measurements. Then, the active molecule was tested in an enzymatic assay aimed at evaluating its capability to inhibit the HIV-1 reverse transcriptase (RT) activity *in vitro*. Because a serious concern in the long-term clinical management of HIV disease is the development of drug resistance that often appears during HAART therapy, reducing its effectiveness. The inhibitory activity of the new biscoumarin was also tested against a panel of viruses possessing mutations that confer selective resistance either to nucleoside (NRTI) and non-nucleoside (NNRTI) RT inhibitors.
Results: The $CHCl_3$ extract displays substantial anti-HIV-1_{IIIB} (EC_{50} = 0.4 µg/ml) activity associated with a moderate cytotoxicity (CC_{50} = 6 µg/ml) against the uninfected MT-4 cells. The new biscoumarin shows a relevant activity (EC_{50} = 8.7 µM) associated with a lower cytotoxicity (CC_{50} = 54 µM). It was observed that the concentration of the active compound did not affect the TEER over the time of the experiment, keeping values similar to those of the untreated cells**.**
Interestingly, the new biscoumarin turned out to be active against resistant strains (EC_{50} ranging from 6.6 to 12.0 µM) but it was not able to inhibit the RT function.
Discussion and Conclusions: In our studies, the newly identified biscoumarin was shown to be active against HIV-1_{IIIB} as well as HIV-1 variants carrying clinically relevant mutations related to NNRTI and NRTI resistance. The safety profile showed that the compound did not affect the TEER and then the integrity of the monolayer until 144 h post-treatment. These findings are encouraging and studies to clearly identify the mechanisms of action for biscoumarins as new anti-HIV-lead are ongoing, including structural modification which is a powerful tool to increase the potential of a bioactive principle.

76 - Decontamination and reuse of surgical masks during COVID-19 pandemic

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Introduction: Experiences during pandemics in the 2000s caused by respiratory viruses, as well as the current COVID-19 pandemic caused by SARS-CoV-2, have highlighted concerns about the pressures that pandemics may have on surgical mask supplies globally, as well as their environmental impact. Decontamination of surgical masks has been proposed as a solution to support the reuse of masks, indeed an increasing number of literature data is analyzing the effectiveness of disinfection of contaminated surgical masks but also the impact of the decontamination process on their performance. Many of these proposed methods, use existing equipment that may already be available in hospitals and could be reused for the decontamination of surgical masks. Some methods can also be applied on household equipment, broadening the usefulness of surgical mask decontamination in a wide range of healthcare settings. To enable the safe reuse of single use surgical masks, it is essential to develop efficient and easy methods to remove persistent microbial contaminants on used masks without damaging bacterial filtration properties. **Materials and Methods:** Four decontamination methods have been tested for treatment of surgical masks, specifically: 1. water immersion at 40°C and 80°C; 2. autoclave steam; 3. microwave generated steam and; 4. ultraviolet germicidal irradiation (UVGI). Breathability and Bacterial Filtration Efficiency (BFE) tests were also performed. **Results:** The preliminary results have shown that all decontamination methods tested allow to obtain a considerable reduction of the microbial load on the surgical masks. **Discussion and Conclusions:** These decontamination methods appear to reduce the risk of the mask as a source of infection for the wearer. Furthermore, the selected methods guarantee that the surgical masks, even after the decontamination procedures, have adequate BFE and breathability values. Further studies will be needed to confirm security of these mask decontamination methods in order to be reused with a view to greater environmental sustainability and greater security of supply.

129 - Prevalence of bacterial and fungal coinfections in COVID-19 patients in a Teaching Hospital of Southern Italy

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Introduction: Coinfection with bacteria and fungi in SARS-CoV-2 is of particular importance due to the possibility of increased morbidity and mortality. These coinfections are frequent in hospitalized patients and isolation of pathogens such as *Klebisella pneumoniae* and *Acinetobacter baumannii complex* is crucial for therapeutic success. In this study, we evaluate the prevalence of bacterial and fungal coinfections in SARS-CoV-2 positive patients.

Materials and Methods: We enrolled a total of 309 SARS-CoV-2 positive patients admitted to University Hospital of Catanzaro, Italy, between March 1st, 2020 and April 30, 2021. Diagnosis of SARS-CoV-2 was confirmed by reverse Real-Time Polymerase Chain Reaction (rRT-PCR) detecting N, E and RdRp genes. Co-infections were confirmed by culture isolation of respiratory and blood samples. Antibiotic resistance was carried out by conventional and molecular assays.

Results: The 309 SARS-CoV-2 positive patients were 177/309 males (57.28%) and 132/309 females (42.72%), with a median age of 67 (± 14.74 SD). In 47/309 (15%) coinfections were detected. In particular, 10/47 patients (21%) developed co-infections from bloodstream, 19/47 patients (41%) from respiratory samples and 18/47 (38%) from both samples. The most frequent pathogens isolated from blood cultures were *Acinetobacter baumannii complex* and *Klebisella pneumoniae* (47% and 26%, respectively), while *Candida albicans* was the most prevalent microorganism from respiratory samples (37%).

Discussion and conclusions: Our data confirm that COVID-19 positive patients were a high-risk category to develop nosocomial and opportunistic infections. Therefore, early and timely diagnosis of co-infections could improve implications of the clinical management of SARS-CoV-2 positive patients.

11 - Intestinal organoid modeling for intestinal bacteria competition assay

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Introduction: The human gastrointestinal (GI) tract is colonized by a complex ecological community of microorganisms, "microbiota", responsible for vital functions, including the induction of immune system development, regulation of intestinal barrier integrity and synthesis of crucial molecules for human health. Pathogens, opportunistic pathogens and pathobionts are able to colonize the intestinal tract by outcompeting the resident flora through the expression of specific phenotypes. On the other hand, the role of beneficial strains, such as probiotics, is to restrain invading pathogens from conquering a specific niche, thereby improving human health. Intestinal organoids represent an "in vivo like" model of the intestinal epithelial surface where this competition battle is played. Hence, we compared the efficacy of the probiotic *E. coli* strain Nissle 1917 (EcN) to prevent enteropathogenic *E. coli* (EPEC, strain E2348/69) infection in intestinal organoids. Materials and methods: Time course infection experiments were performed on 2D and 3D cultured mouse intestinal organoids. At the selected time points organoids were either lyzed for bacterial counting or stained for fluorescence experiments. Results: Bacterial time-course co-infection experiments revealed the enhanced capability of the Nissle strain to adhere to the cells to inhibit EPEC attachment when the bacteria were in contact with the apical side of the epithelium (2D model). Vice versa, the 3D model highlighted and an unexplored ability of EPEC to contact the intestinal tissue from the basal side and this interaction was not inhibited by the presence of Nissle. Discussion and conclusions: Results demonstrated that intestinal organoids are the most suitable in vitro model to test the efficiency of specific probiotic strains in protecting intestinal tissues from invading pathogens. Moreover, they offer the possibility to unveil new virulence traits enabling intestinal pathogens to interact with the basal side of the epithelium.

113 - Modulation of miRNome by HCMV and HHV-6 infection in human dermal fibroblasts: possible significance in systemic sclerosis.

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Introduction: Human cytomegalovirus (HCMV) and Human herpesvirus 6 (HHV-6) have been reportedly suggested as triggers of the onset and/or progression of many autoimmune diseases. Among them, we recently reported a possible involvement of both viruses in systemic sclerosis (SSc), a severe autoimmune disorder characterized by vasculopathy and multi-organ fibrosis. The etiology and pathogenesis of SSc are still largely unknown but virological and immunological observations support a role for these beta-herpesviruses. We recently observed a direct impact of HCMV and HHV-6 infection on the expression of cell factors associated with fibrosis at the cell microenvironment level. Since in SSc patients miRNA expression has been found deregulated at the tissue or blood level, here we aimed to investigate the impact of HCMV and HHV-6 infection on the miRNome of *in vitro* infected primary human dermal fibroblasts, which represent one of the main SSc target cells.
Materials and Methods: Human primary dermal fibroblasts were infected in vitro with cell-free inocula of HCMV and HHV-6, and at different times post infection (0, 4, 7, 10, and 14 d.p.i.) were collected to extract RNA. The analysis was performed by Taqman arrays detecting and quantifying 754 miRNAs.
Results: The miRNome analysis showed that both viruses significantly modulated miRNA expression in infected cells, with effects evident at both early and late times p.i.. PCA analysis showed a significantly different clusterization of miRNA at all time tested. Up to 106 miRNAs were up-regulated and 170 down-modulated by HCMV infection; HHV-6 infection up-regulated the expression of up to 117 miRNA and down-modulated 112 miRNAs. Several altered miRNAs belong to those already recognized for their key function in fibrosis; several other miRNAs appear potentially involved in the process leading to cell function impairment and apoptosis.
Discussion and Conclusions: HCMV and HHV-6 infection profoundly remodulate cell miRNome in human dermal fibroblasts, and the correlation between these *in vitro* results with *in vivo* observations is strongly suggestive of a role of HCMV and HHV-6 in the multistep pathogenesis of fibrosis in SSc.

88 - Longitudinal evaluation of the IgA, IgG and neutralizing antibodies in a pediatric population mildly affected by SARS-CoV-2 infections

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1. Introduction

Children generally develop a mild disease after severe acute respiratory syndrome (SARS) CoV-2 infection. The exact mechanisms that protect them from severe COVID-19 disease are yet to be defined; a reason may be a strong and effective innate and/or adaptive immune response to SARS-CoV-2. Both asymptomatic and symptomatic SARS-CoV-2 infections elicit polyfunctional antibodies (Ab), lower in asymptomatic than in symptomatic adults and at very high levels in severe COVID-19 patients. Neutralizing Ab are the most important type of Ig that control viral infections. To gain insight into Ab responses to SARS-CoV-2 in children we measured levels of serum IgA, IgG and neutralizing (nt) Ab in sequential samples from children that were evaluated also for type of respiratory symptoms and co-morbidities.

2. Materials and Methods

Children and adolescents (aged 0-19 years) attending pediatric ambulatories Dpt, Sapienza University of Rome, were consecutively enrolled (September-March, 2021). Those who were positive to SARS-CoV-2 infection through PCR on nasopharyngeal swabs returned after 20-30 (T1) and 30-60 (T2) days from the first positive sample to acquire a blood venous sample. Anti-Spike IgG and IgA were measured using ELISA kits (Euroimmun) and levels expressed as RU. Nt Ab were tested using replication-competent VSV-pseudovirus (kind gift from J. Hiscott, Institute Pasteur, Rome) expressing the SARS-CoV-2 spike protein and titers were calculated as $TCID_{50}$. Statistical analysis was performed using SPSS.27.

3. Results

A total of 115 children (mean age: 11,5 years, range 1-19 years) were enrolled and provided serum at T1 and T2. IgA at T1 were significantly higher than at T2 ($p < 0,001$) whereas IgG and nt Ab were not different. Stratifying children by age-class (1-8, 8-14 and 14-19 years old) we found significative differences in IgA, IgG and nt Ab levels among classes ($p < 0,05$) with higher levels in younger children; interestingly, in children that were 14 years and older, IgA, IgG and nt Ab level were lower comparing to the younger age class ($p < 0,05$) both in T1 and T2. Participants were then stratified in 3 groups (no symptoms, 1-2 symptoms, more than 2 symptoms); adolescents were more represented

in the group with more symptoms. In T2 samples, children with no symptoms had higher levels of IgA with respect to children with more than 2 symptoms (p = 0,018) but IgG did not differ.

4. Discussion and Conclusions

This study showed robust Ab responses for at least two months after SARS-CoV-2 infections that were higher in asymptomatic/mildly affected children than in adolescents. If confirmed in larger groups, these data would suggest that asymptomatic/mild SARS-CoV-2 infections would elicit Ab levels protective from reinfections.

12 - Compounds released from Lactobacillus (L.) acidophilus, L. plantarum, L. rhamnosus and L. reuteri inhibit Candida parapsilosis pathogenic potential after infection of vaginal epithelial cells in vitro

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INTRODUCTION. *Lactobacillus spp*. are the most represented microorganisms in the vaginal microbiota of healthy women, where they provide a shelter against infections from several pathogens, such as the yeasts belonging to the genus *Candida*. The latter are responsible for the vulvovaginal candidiasis (VVC), a condition affecting up to 75% of women during their child-bearing age at least once in their lifetime. Moreover, 5-8% of such women develop the recurrent form of the disease (RVVC), consisting of at least 5 VVC episodes per year. Notwithstanding *C. albicans* is the main responsible of VVC cases, in the last decades, the incidence of VVC cases by non-*albicans Candida* (NAC) species has become prevalent, especially in some geographical areas. *C. parapsilosis*, in particular, has been reported to be second species most commonly isolated from women affected by VVC. However, little is known on this species, and on its role in the pathogenesis of VVC.

MATERIALS AND METHODS. Cell-free supernatants (CFS) were obtained following an overnight culture of 4 different Lactobacilli species (*L. acidophilus, L. plantarum, L. rhamnosus, L. reuteri*). Lactobacilli-released compounds, contained in CFS, were assessed for their effect on several virulence factors of *C. parapsilosis* (strain CLIB214), such as growth rate, capacity to form pseudohyphae, capacity to adhere to a vaginal epithelium *in vitro* (A-431 cells monolayer) and to induce cell damage. The latter was evaluated by measuring lactate dehydrogenase (LDH) release from A431 cells.

RESULTS. *C. parapsilosis* growth inhibition by *L. acidophilus*, *L. plantarum* and *L. reuteri* CFS was 47%, 55% and 52% respectively, whereas *L. rhamnosus* CFS effect was weaker (33% inhibition growth). All the Lactobacilli significantly inhibited *C. parapsilosis* adhesion to vaginal epithelial cells: upon incubation with CFS, only 5-7% of fungal cells adhered to epithelial cells, after 90 minutes incubation; differently, the adhesion of the control reached 19%. Interestingly, no effect on pseudohyphae formation by any of the CSF was ever observed. Finally, the *C. parapsilosis*-induced damage on A-431 cells was significantly reduced by the addition of the CSF.

DISCUSSION AND CONCLUSIONS. Our results show that the investigated species of Lactobacilli release compounds capable to impair several *C. parapsilosis* virulence factors, such as growth rate and adhesion to vaginal epithelial cells; interestingly, while not affecting fungal capacity to form pseudohyphae, such compounds significantly reduce Candida-mediated epithelial damage.. These data suggest that, in the context of vaginal microbiota, these Lactobacilli species may play an important role in counteracting the onset of mucosal *Candida* infections.

109 - Trikafta as a final frontier in CF patients: clinical and microbiological implications

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Introduction. Elexacaftor-tezacaftor-ivacaftor is a recently approved triple combination therapy for the modulation of *Cystic Fibrosis Transmembrane Conductance Regulator* (CFTR), containing two correctors and a potentiator of the channel. In June 2021, its use was approved in all Italian patients aged 12 years and older with one F508del mutation and one minimal function mutation (F/MF) or two F508del mutations (F/F) in the *CFTR* gene. In Italy, triple combination therapy was already approved as compassionate use before this date. We present a one-year case-control study of 26 patients enrolled at Palermo Regional Reference Center for Cystic Fibrosis.

Materials and Methods. All recruited patients were 18 years and older. 13 of them had at least one copy of the F508del mutation: in particular, 5 were homozygous for the above mutation (F/F), 5 were heterozygous and 3 had an F508del mutation and a minimal function mutation (F/MF). This group of patients had the worst clinical condition and those with a predicted forced expiratory volume in 1 second (FEV1) of 40% or less were enrolled in the study and received elexacaftor-tezacaftor-ivacaftor combination therapy. The remaining 13 patients had less critical genotypes and better clinical condition, so they did not receive any treatment. Sputum samples were collected from all patients in the two groups. The samples were inoculated into enriched and selective agar media after dilution. The isolated microorganisms were identified by MALDI-TOF *Bruker*, while the susceptibility tests were performed using BD Phoenix or Microscan *Walkaway*.

Results. A number of 120 strains were collected and divided into: *S. aureus* (50), *P. aeruginosa* (38), *A. niger* (1), *A. xylosoxidans* (5), *C. albicans* (1), *C. freundii* (1), *C. lusitaniae* (1), *C. parapsilosis* (1), *E. cloacae* (1), *E. coli* (3), *K. pneumoniae* (2), *P. mirabilis* (1), *S. maltophilia* (1) and *S. pneumoniae* (1). Only 2 patients among those treated had respiratory exacerbations in twelve months; overall, all showed a significant reduction in airway colonization. 41.5% of samples collected in the treated group showed complete negativity of sputum (**P <0.05**), in contrast to the untreated patients who had recurrent respiratory colonization and consistently positive sputum samples.

Discussion and conclusions. While the clinical and instrumental benefits of elexacaftor-tezacaftor-ivacaftor combination therapy are well established, further studies are needed to investigate how these drugs, which alter the properties of airway mucus, can lead to a significant reduction in microbial colonization and subsequently a reduction in pulmonary exacerbations in patients with cystic fibrosis.

123 - Role of NRF2, G6PD and APE1 in the regulation of influenza virus replication and virus-induced inflammatory response

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Introduction: The imbalance of the redox-state plays a pivotal role in promoting viral replication and the inflammatory response to viral infection, including that by influenza virus. One of the most important regulators of the cellular redox-state, the nuclear factor erythroid-2 related factor 2 (NRF2) is found to be downregulated during the influenza virus infection, although the mechanisms at the basis of this downregulation are not well defined. NRF2 regulates the expression of many anti-oxidant factors, including glucose-6-phosphate dehydrogenase (G6PD), which is crucial to restore the NAPDH levels and to regenerate glutathione (GSH), the main intracellular antioxidant. The NRF2 pathway regulates also the expression of the NLRP3 inflammasome, which is important to fight the viral infection, but it could be detrimental if its activation becomes uncontrolled. Between the modulators of NRF2 there is the Apurinic/apyrimidinic endonuclease/Redox-factor 1 (APE1/REF-1). Some authors report a positive interaction between the two proteins, others show that APE1 redox function can repress the NRF2 activation, however whether it contributes to the regulation of the antioxidant response during influenza virus infection is still unknown.

Methods: A549 cells were infected with influenza A/Puerto Rico 8/34 H1N1 (PR8) virus. To characterize the expression of NRF2, APE1 and NLRP3, the cellular pellet was collected at 24 hours post infection to perform Western Blot and RT-PCR. The silencing of G6PD was performed one day before the infection.

Results: Preliminary data showed that, compared to mock-infected cells, NRF2 RNA levels were downregulated by 40% in infected cells and by 70% in G6PD-silenced infected cells, while APE1 levels were upregulated by 40% in infected cells and downregulated by 25% in G6PD-silenced cells. Accordingly, NRF2 protein expression was downregulated by 63% in infected cells and 67.5% in G6PD silenced cells, while APE1 nuclear protein levels were downregulated by 33% in infected cells and by 53% in G6PD-silenced cells. The RNA levels of NLRP3 and pro-inflammatory cytokine IL-6 were respectively upregulated by 3.7 and 18 folds in infected cells and 5 and 34 folds in G6PD-silenced infected cells.

Conclusions: Overall the results indicate a negative feedback between the NRF2 protein levels and APE1 mRNA expression during influenza virus infection. The fact that also NLRP3 RNA levels are increased during infection confirms a strong activation of inflammatory response, especially in G6PD-deficiency conditions, although it remains to evaluate the role of APE1 pathways in the control of NRF2 and in the activation of gene related to antioxidant response. Further studies are in progress to clarify its role in the pathogenesis of the influenza virus.

99 - Evaluation of dynamic conditions in biofilm growth using two bioreactors with different shear rates in the study of device-related infections.

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Introduction: Device-related infections are the third leading cause of prosthetic implant failure in primary surgery and is the leading cause in revision surgery within the first five years. Aim of this study was to generate biofilm under static and dynamic conditions to evaluate the most suitable orthopedic materials on the prevention of device-related infections.

Method: Biofilms of *Staphylococcus epidermidis* (ATCC 35984) and *Pseudomonas aeruginosa* (ATCC 700888) were generated under static and dynamic condition with the Drip Flow Reactor (DFR) and the CDC Biofilm Reactor (CBR). In the bioreactors were housed coupons of different orthopedic uncoated and coated materials. After 48h biofilm was determined quantitatively by the MTT colorimetric assay and according to the ASTM E2647-13. The expression of genes involved in Quorum Sensing was studied by comparing the different expression between the QS gene expressed by *P. aeruginosa* during biofilm formation (lasR, rhlR) and a reference housekeeping (rpoD) gene constitutively expressed in all cells.

Results: Dynamic model showed a better capacity of *S.epidermidis* to grow with a rotation between 60-120 rpm on each tested materials (Mann-Whitney test, p-value < 0,05) than *P.aeruginosa*. Titanium was the material on which the bacterial strains adhered less, whereas carbon and polycarbonate allowed greatest adherence of *P.aeruginosa* (Mann-Whitney test, p-value < 0,05). Results of static model showed that both species grew on each materials without distinction (Kruskal-Wallis test, p-value 0,95). Comparing data obtained by relative quantification, it was shown that silver nanoparticles increased the expression of rhlR gene 2.7 fold on coated titanium versus uncoated.

Conclusions: The static model was not able to evaluate the adhesion capacity of the different strains, confirming the dynamic model as the most suitable tool for the study of orthopedic materials. The silver nanoparticles were found to be able to positively regulate the expression of the *P. aeruginosa* QS regulator gene rhlR.

36 - Preliminary investigation on the volatilome of Streptococcus pyogenes SF370

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Introduction

The objective of the study was to identify microbial volatile compounds (mVOCs) produced by *Streptococcus pyogenes*, one of the most common human pathogens, and to attempt the determination of its minimal volatilome. In recent years, the characterization of this subset of the bacterial metabolome ha attracted specific interest also in view of the possibility of exploiting specific mVOCs as potential biomarkers of an ongoing bacterial infection.

Materials and methods

General preparation and analysis of samples were done following procedures described for the study of the *Staphylococcus aureus* volatilome. The *Streptococcus pyogenes* strain was the SF370. We performed a triplicate of the *S. pyogenes* culture and a triplicate of the medium (Todd Hewitt + 0.2% yeast extract, THY) incubated under the same conditions as the bacterial culture. Samples were taken at mid-log and in the pre-stationary phase of the growth curve and centrifuged. Supernatants were then filtered. The mVOCs were extracted using a mixed fiber and subjected to Gas Chromatography/Mass Spectrometry (GC-MS) analysis. GC separation was accomplished using a DB-WAX polar column (60 m x 0.25 mm x 0.00025 mm). The compounds were identified by comparison with the NIST database and with published retention indices.

Results

The analysis of all profiles showed a good intra-sampling reproducibility. The comparative analysis of the samples allowed groups of compounds to be excluded from further analysis based on: (i) presence in all the experimental conditions or (ii) presence in only one of the replicates or (iii) poor identification score. The results showed that metabolic activity of *S. pyogenes* grown in THY produces mVOCs which are more polar than the volatiles present in the uncultured medium. At mid-log, the minimal volatilome consisted of 2-nitroethanol, 2-tert-butoxyethanol, 1-butanol, 3,3-dimethylhexane, pentadecane, 10-methylnonadecane and 2-tridecanone while ethanol, 1-butanol and gamma-aminobutyric acid were characteristic of the pre-stationary phase.

Conclusions

Thanks to this preliminary study, it has been possible to determine the minimum volatilome of *S. pyogenes* strain SF370 grown in THY. Further studies using different culture media, different clinical strains of *S. pyogenes* and analytical conditions (e.g. column and fiber type) are needed to broaden the definition of the pool of volatile molecules produced by this bacterial species.

97 - Immunomodulatory properties of lipopolysaccharides isolated from marine bacteria Echinicola pacifica and Echinicola vietnamensis

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Introduction: Lipopolysaccharides (LPSs) are widely known for their central role in many aspects of host-microbe interaction events. In particular, its glycolipid component, the lipid A, is recognized by the mammalian innate immune receptor complex composed by myeloid differentiation protein-2 (MD-2)/Toll-like receptor 4 (TLR4) and acts as strong stimulator of inflammatory response. Some lipid A exhibit a weak or no immunopotency and, in some cases, an inhibition of the toxic effects of pathogenic LPS on TLR4/MD-2 expressing cells that can be of inspiration for development of an alternative approach in the treatment of inflammatory disorders. In Gram-negative bacteria residing in extreme habitats, including marine bacteria, are commonly observed lipid A structural modifications associated with the need to maintain cell envelope rigidity and integrity upon variations of the surrounding environments. Here, we have focused our attention on immunomodulatory activity of LPSs isolated from two marine bacteria belonging to *Echinicola* genus, *E. pacifica* KMM 6172 and *E. vietnamensis* KMM 6221 by measuring NF-*k*B signaling in an engineered human TLR4 reported cell model (HEK-Blue hTLR4). **Materials and Methods:** HEK-Blue hTLR4 cells were seeded in a 96-multiwell plate at a density of 3×10^4 cells/well. The activation assay was conducted stimulating cells with different concentrations (1, 10, 100 ng/ml) of *E. pacifica* KMM 6172 LPS, *E. vietnamensis* KMM 6221 LPS or *Salmonella typhi* SH 2201 LPS for 16 h by evaluating NF-*k*B-dependent secreted alkaline phosphatase (SEAP). While, to evaluate the ability of *E. pacifica* KMM 6172 LPS or *E. vietnamensis* KMM 6221 LPS to interfere with the TLR4-mediated signalling triggered by the *S. typhi* LPS, the competition assay was performed preincubating cells with *E. pacifica* KMM 6172 LPS or *E. vietnamensis* KMM 6221 LPS (1, 10, 100 ng/ml) for 90 min and then adding 1 ng/ml of *S. typhi* SH 2201 LPS. After 16 h of incubation SEAP-containing supernatants were analyzed. SEAP levels (OD at 620nm) were used as indicator of TLR4 pathway activation. The same procedures were performed with HEK-Blue hTLR2 cells and HEK-Blue Null2 cells, as controls. **Results:** HEK-Blue hTLR4 cells incubated with *E. pacifica* KMM 6172 LPS or *E. vietnamensis* KMM 6221 LPS produced moderate amounts of SEAP compared to *S. typhi* SH 2201 LPS significant stimulation. Our results clearly showed that *E. pacifica* KMM 6172 and *E. vietnamensis* KMM 6221 LPSs significantly reduced *S. typhi* LPS-dependent TLR4-mediated NF-*k*B activation. **Discussion and Conclusions:** The data suggested interesting inhibitory properties of *E. pacifica* and *E. vietnamensis* LPSs against toxic effect of *S. typhi* LPS highlighting a potential immunomodulatory role of unexplored LPSs.

9 - Grape canes from typical cultivars of Campania as a source of high-value bioactive compounds: phenolic profile, antioxidant and antimicrobial activities

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Introduction. In recent years, the interest in the use of agro-industrial wastes as natural resources with high added value has grown considerably. The wine production process generates a great amount of solid residues (peel, cane, stalk and seed), and grape canes are the main solid waste from vineyards. Currently, this waste is hardly used, and its exploitation could represent a new and economical source of bioactive compounds. In the present study, a simple extraction method was applied for the recovery of phenolic compounds from grape canes belonging to the three typical Campania cultivars, "Aglianico", "Fiano" and "Greco", using water as an extraction solvent at different pH. The total content of phenols, flavonoids, orthophenols and tannins was determined in the extracts and the antioxidant activity was also evaluated. Finally, the antimicrobial activity of the extracts against bacteria, viruses and fungi that cause human infections was also investigated.

Materials and Methods. Aqueous extracts at different pHs (1–13) were prepared from “Aglianico”, “Fiano”, and “Greco” grape canes. The Radical Scavenging Activity (RSA) and the Ferric Reducing Antioxidant Power (FRAP) were measured. Finally, the antimicrobial activity was evaluated against bacteria (*Staphylococcuc aureus* and *Escherichia coli*), fungi (*Candida albicans*) and viruses (Herpes simplex virus type 1 and 2). **Results.** The alkaline pH (13.00) produced the best polyphenol-rich extracts, as the total phenolic content was more than double when compared to the respective extracts prepared at pH 1.00. “Greco” grape canes gave the highest quantity of phenolic compounds at each pH, and they showed also the highest antioxidant activity at pH 7.00. Seventy-five compounds were identified in the extracts by HPLC-MS with six of them described for the first time in grape canes. Procyanidins were highly abundant in extracts at pH 7.00, whereas stilbenoids were the most represented compounds at pH 13.00. Very strong antiviral activity against herpes simplex viruses was recorded for the extracts at pH 7.00 and 13.00 that were active in the early stages of infection by acting directly against the viral particles.

Discussion and Conclusions. The valorisation of grape canes, a by-product of vine processing produced in large amounts, was achieved through the production of active aqueous extracts. The importance and dimension of the wine industry worldwide justify the need to redirect this waste, currently not valued, to more significant and environmentally friendly uses. The overall results suggest that grape canes, currently underutilized, can be usefully valorised by providing active extracts to use as antioxidant and antiviral agents.

152 - Detection of SARS-CoV-2 RNA and evaluation of neurofilament light chain in cerebrospinal fluid and plasma samples of COVID-19 patients

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Background

Neurofilament light chain (NfL) is considered a specific biomarker of quantitate neuro-axonal damage and normally measured in CSF. Novel methods have given the possibility to measure NfL in plasma instead. Here, we investigated SARS-CoV-2 RNA presence in CSF and plasma samples in COVID-19 patients with neurological symptoms using droplet digital PCR (ddPCR). Moreover, on CSF and plasma samples we assessed NfL levels as well as matrix metalloproteinase-9 (MMP-9), which contributes to blood barrier brain damage, and its specific inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1) evaluating the association with disease severity.

Materials and methods

Hospitalized COVID-19 patients with neurological symptoms were enrolled. Plasma and CSF samples were drawn at the acute stage of disease. Using ddPCR viral RNA detection and quantification in CSF and plasma samples were performed. NfL evaluation was assessed using the Simple PlexTM Ella (Ella^{TM}) microfluidic platform. Finally, CSF and plasma levels of MMP-9 and TIMP-1 were evaluated by ELISA. According to the ARDS onset, COVID-19 patients were stratified into ARDS group and non-ARDS group. As control group, we enrolled healthy donors (HD) matched for gender and age to compare NfL, MMP-9 and TIMP-1 plasmatic levels.

Results

Twelve COVID-19 patients with neurological symptoms and 13 HD were enrolled. In CSF and plasma samples viral RNA was detected in 4/12 and 1/12 COVID-19 patients, respectively. According to SARS-CoV-2 detection in CSF samples, not statistically significant differences in NfL, MMP-9 and TIMP-1 levels were found. Otherwise, ARDS group (n=6) showed higher NfL levels and lower MMP-9/TIMP-1 ratio on CSF samples compared to non-ARDS group (n=6) (NfL: 6480 [1512-11012] and 476 [305-2859], p=0.026; MMP-9/TIMP-1 ratio: 0.5 [0.3-0.6] and 0.9 [0.6-0.9], p=0.036).

A positive correlation between NfL levels on CSF and plasma samples (ρ=0.810 p=0.022) was observed. Furthermore, a negative correlation between NfL levels on CSF and MMP-9/TIMP-1 ratio on CSF (ρ=-0.631 p=0.032) was observed.

Finally, COVID-19 patients showed significantly higher plasma levels of NfL, MMP-9 and TIMP-1 compared to HD (NfL: 72 [28-95] and 11 [9-17] pg/ml, p<0.0001; MMP-9: 192 [74-268] and 51 [34-70], p=0.0017; TIMP-1: 319 [201-395] and 61[33-70], p<0.0001). A significantly lower plasma MMP-9/TIMP-1 ratio in COVID-19 patients compared to HD was observed (0.6 [0.4-0.7] and 1.0 [0.6-1.6], respectively, p=0.034).

Discussion and conclusion

Direct invasion of the CNS by SARS-CoV-2 is a controversial issue, with contradictory findings in current literature. As suggest by our data, ARDS is associated to CNS damage and neurological sequelae also in the absence of SARS-CoV-2 detection in CSF. Our data corroborated the clinical relevance of NfL and MMP-9/TIMP-1 in COVID-19 induced neural damage. NfL, MMP-9 and TIMP-1 evaluation on plasma samples can be useful to detect and monitor CNS damage in COVID-19.

02 Immunità e vaccini

128 - Monitoring anti SARS-CoV-2 humoral and cell-mediated immune response after BNT162b2 vaccine.

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Introduction: We have designed a prospective study with the aims to explore and monitor the antibody response against SARS-CoV-2 virus induced by the COMIRNATY mRNA vaccine in a sample of 178 vaccinated volunteers.
Here, we report the interim analysis of the study, including serological results ten days, thirty days, three months and six months (in process) after the second mRNA BNT162b2 vaccine administration among healthcare personnel at 'S. Maria alle Scotte' University Hospital in Siena. For each time point, 60 subject's sera were also tested for the presence of specific neutralizing antibodies. Moreover, at six months, peripheral blood mononuclear cells (PBMC) from 20 subjects, equally distributed between those having a high and low humoral response, were cultured and stimulated *in vitro* with recombinant SARS-CoV-2 Trimeric Spike protein in order to analyze the cellular immune response, especially B and T cells memory subsets, by FACS analysis.
Materials and Methods: The humoral immune response of all healthcare workers was evaluated by chemiluminescent assay (CMIA) and, among them, 60 were also tested by live virus-based neutralization assay. For FACS analysis, $1x10^6$ PBMC were cultured in 24-well plates and stimulated with the recombinant Spike protein (Leinco Technologies). Then, T and B cell subsets were analyzed 24 and 96 hours later respectively. Moreover, three samples of uninfected, non-vaccinated subjects were included in the study as negative controls.
Results: Circulating IgG levels declined between ten and thirty days after vaccination, despite neutralizing titers remained quite stable, while a robust decrease of both circulating and neutralizing antibodies was measured both three ($p<0.05$ respect to thirty days) and six months ($p<0.05$ respect to three month) after vaccination**.** The role of cellular immune response is still under investigation.
Discussion and Conclusions:
These findings provided evidence of the decline of both circulating and neutralizing anti-SARS-CoV-2 antibodies three and six months after receiving the second dose, thus raising questions about the need for a booster dose. Results of FACS analysis, which are under investigation, will be useful to understand the role of B and T cells responses in vaccinated subjects after being re-exposed to the antigen.

10 - EMERGENCE OF SARS-COV-2 SPIKE ESCAPE MUTATION Q493R AFTER BAMLANIVIMAB/ETESEVIMAB TREATMENT FOR COVID-19

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Keywords: SARS-CoV-2; COVID-19; variant of interest; Spike; receptor-binding motif; Q493R.

SARS-CoV-2 variants are usually a consequence of random mutations in humans or other hosts, but accelerated evolution can also occur under selective pressure from therapeutic interventions with neutralizing antibodies[1]. Bamlanivimab has been recently withdrawn from the vendor as a monotherapy because of failure against E484K SARS-CoV-2 variants. Emergency use remains authorized for the bamlanivimab/etesevimab cocktail [2], for which no completely resistant variant has been reported to date. Accordingly, such cocktail has shown effective at reducing hospitalizations when administered early after infection [3]. Given mounting reports of accelerated intra-host evolution of resistant SARS-CoV-2 clades following neutralizing antibody-based treatments [1,4-7], we started screening patients who failed to negativize the nasopharyngeal swab (NPS) after bamlanivimab/etesevimab treatment.

We report here the first *in vivo* case of a Spike escape mutation conferring combined resistance to both bamlanivimab and etesevimab.

A 73-years old male was diagnosed on February 2021 with cholangiocarcinoma: while waiting for chemotherapy, he developed sepsis and was admitted to Varese hospital on Apr 12 for steroid and antimicrobial treatment. Follow-up NPS remained positive on Apr 28 (Ct 15) and May 3 (Ct 24); a chest CT scan on Apr 30 showed progression to interstitial pneumonia, and the patient was placed in noninvasive ventilation. No further bamlanivimab/etesevimab infusion was performed.

According to national guidelines, SARS-CoV-2-positive samples were sequenced. Spike gene sequencing on the Apr 24 NPS revealed a PANGOLIN clade B.1.1.7. Since May 3 we observed a secondary A1478G peak in the *S* gene, corresponding to the Spike Q493R mutation, which became predominant as soon as May 8.

E484, F490, Q493, and S494 are the 4 amino acid residues within the Spike receptor-binding motif (RBM) that are known to be critical for bamlanivimab binding. Q493 is also among the many more RBM residues crucial for interactions with etesivimab. Q493R/K (which can be selected *in vitro* by bamlanivimab[8]) is to date the only mutation that causes resistance to both bamlanivimab and etesivimab.

In conclusion, we have shown here that mutations conferring resistance to both bamlanivimab and etesevimab can arise *in vivo*: Q493 mutations increase binding affinity to ACE2 [10], but further studies are needed to clarify whether such escape mutants are fit enough to spread and persist in humans.

148 - Virological clearance profile in course of treatment with anti-SARS-CoV-2 monoclonal antibodies in patients diagnosed with mild-moderate COVID-19

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Introduction Anti-SARS-CoV-2 neutralizing monoclonal antibodies (mAbs) have shown efficacy and good tolerability in high-risk outpatients with mild-moderate COVID-19. AIFA indicated the treatment with mAbs in non-hospitalized patients with at least one risk factor for disease progression within 10 days from symptom onset. Data about real-life settings are still lacking. We here report data about efficacy and safety of anti-SARS-CoV-2 mAbs in a cohort of high-risk patients in Milan. We aimed at exploring the association between baseline nasopharyngeal (NP) viral load and virological clearance on the 7th day after treatment.

Materials and methods

We consecutively enrolled high-risk patients with PCR-confirmed mild-moderate COVID-19. High-risk patients were defined according to AIFA indications for treatment with mAbs, i.e. BMI ≥35, dialysis, diabetes, immunodeficiencies, age ≥55 years with cardio-cerebrovascular or chronic pulmonary diseases. Patients were treated with bamlanivimab 700 mg, bamlanivimab 700 mg/etesevimab 1400 mg or casirivimab 1200 mg/imdevimab 1200 mg in a dedicated ID outpatient service. Clinical recovery was defined as absence of fever and S02 >95% in room air, while virological clearance was declared with one PCR-negative NP swab. We collected NP swab, saliva and plasma at t0 (infusion) and after 7 days (t7). Routine blood tests were also performed. SARS CoV-2 viral load was measured on specimens by quantitative RT-PCR. Chi-square, Mann-Whitney, Wilcoxon tests for paired samples and a logistic regression model were used for the statistical analyses.

Results

69 patients were included; of these 45 (76%) reached clinical and virological clearance within 7 days. 8 (11.6%) patients received bamlanivimab as monotherapy, 32 (46.4%) bamlanivimab/etesevimab, and 29 (42%) casirivimab/imdevimab. Median time from symptom onset to clinical recovery was 12 (IQR 9-14) days. mAbs showed efficacy in 64/69 (92.7%) patients; 2 (2.9%) patients were hospitalized after infusion for fever and respiratory failure: 1 required CPAP therapy and 1 received high-flow oxygen therapy; both patients recovered and were discharged home. Median NP and saliva viral load significantly decreased from t0 to t7. Presence of viremia was observed more frequently in patients presenting with higher viral load at t0 (p=0.0039).

Discussion and Conclusions

Our real-life data confirm that anti-SARS-CoV-2 mAbs can be safely administered in outpatient settings and are effective in reducing disease progression. Higher viral loads on NP swab and viremia are associated with delayed virological clearance despite treatment with monoclonal antibodies. Given the persistence of COVID-19 pandemic, mAbs offer an optimal opportunity for the treatment of mild-moderate COVID-19.

124 - Neutralization of SARS-CoV-2 variants by convalescent and post-vaccine serum

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Introduction

The pandemic caused by SARS-CoV-2 is a public health emergency of international concern.

Neutralizing antibodies (NAbs) against SARS-CoV-2 have been considered efficient therapeutic agents to treat the coronavirus disease 2019 (COVID-19). Nevertheless, the emergence and rapid spreading of viral variants worldwide may potentially limit this therapeutic option. The aim of this study is to evaluate neutralizing activity of serum collected from vaccinated (Pfizer) and convalescent donors against different variants of SARS-CoV-2.

Materials and methods

Serum neutralization test was performed in microtiter plates. This procedure takes 3 days to complete and readout was based on the cytopathic effect (CPE). Different clinical isolates of SARS-CoV-2 were used to detect the neutralization of the virus: the original and Brasilian, Indian, English, Nigerian and Sud-African variants.

Results

The results indicate that NAbs titre against wt SARS-CoV-2 are generally higher in plasma from vaccinated subject compared to convalescents.

Discussion and conclusions

The observation that natural infection induces a generalized lower production of neutralizing antibodies compared to vaccination further supports the importance of mass vaccination campaign. The neutralization potency of vaccinated and convalescent plasma against Brasilian, English, Indian, Nigerian and Sud-African SARS-CoV-2variants is also discussed.

133 - Evaluation of a SARS-CoV-2 neutralizing antibodies test with a commercial platform

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Introduction
The pandemic outbreak of SARS-CoV-2 heavily impacted the healthcare system worldwide. Recent vaccine introduction contributed spread immunization. Serological immunoassays able to detect IgG neutralizing antibodies are of interest in monitoring COVID-19 immunization. The SARS-CoV-2 Neutralization Ab (eCLIA) assay on eCL 8000 Fully Automated ECL Analyzer evaluates the *in vitro* quantitative determination of SARS-CoV-2 ACE2-RBD binding neutralizers in serum. The performance of sensitivity and specificity of eCL test was evaluated in comparison the semi-quantitative ELISA ACE2-RBD SARS-CoV-2 Neutralization Assay test (Alifax, Polverara, Padova, Italy) (ELISA).

Materials and methods
Sensitivity of eCL test was evaluated on anonymous sera from 53 vaccinated volunteers. Among these patients, 39 were selected from vaccinated patients without previous SARS-CoV-2 infection (VNI) and 14 from patients recovered from COVID-19 infection before vaccination (VI). Sera were collected at vaccine administration (T0) and after 14, 21 and 28 days (T14, T21, T28). A total of 39 VNI samples were analyzed at T0 and 11 of them were evaluated also at T14, T21 and T28. Concerning VIs, 14 were collected at T0 and 11 also at T14, T21 and T28. All patients received both doses of BioNTech-Pfizer vaccine. Specificity was evaluated on 10 sera collected in the preCOVID-19 era. The IgG negativity at T0 (38/39) and IgG positivity of VI and VNI cohorts at T14, T21 and T28 (11/11) were confirmed with SARS-CoV-2 IgG routine tests on Architect (Abbott Park, Illinois, USA).

Results
Overall, 151 and 10 SARS-CoV-2 positive and negative samples, respectively, were processed. Sensitivity for VNI samples for eCL and ELISA was 72.7% (8/11) and 54.5% (6/11) at T14, 90.9% (10/11) and 63.6% (7/11) at T21 and 100% at T28 (11/11), respectively. The eCL and ELISA assays presented both 100% of sensitivity for VIs for each times performed. Discrepancy between Nab eCL false negative results and ELISA positive results was 6% (2/33). The VNI T0 specificity was 81.5 % (31/38) for CL and 86,8 % (33/38) for ELISA and 100% for samples collected in the preCOVID-19 era. The overall discrepancy between Nab eCL false positive result and ELISA test negative samples is 25% (9/35).

Discussion and conclusion
The eCL test showed good sensitivity for VNI samples at T14 and at T21 was comparable with the ELISA assay performance. Given suboptimal specificity of T0 VNI, support of eCL Nab assay with diagnostic anti-Spike test would be suggested. Specific neutralization test on cell cultures would be necessary for a complete sensitivity evaluation. As the neutralizing IgG threshold that confers safe immunization to COVID-19 is still unknown, more studies are needed for a better definition of a possible cut-off value.

135 - Performance evaluation of an automated chemiluminescence immunoassay for detection of IgM and IgG of SARS-CoV-2

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Introduction

The SARS-CoV-2 pandemic affected more than 200 million people worldwide. As RT-PCR is considered the gold standard for diagnosis of COVID-19, different serological immunoassays were developed to monitor immunization after infection or vaccination. Automated chemiluminescence immunoassay (CLIA) focuses on detecting serum IgM and IgG antibodies against the coronavirus spike protein (S protein) showing high sensibility and throughput. Among CLIA systems, the SARS-CoV-2 IgM and SARS-CoV-2 IgG assays on 8000 Fully Automated ECL Analyzer (Lifotronic, Shenzhen city) (eCl) device employs the tri-(2,2-bipyridine) ruthenium (II)-based direct ECL method and sandwich assay as a measurement principle in detecting IgM and IgG, respectively. The eCL system, targeting N protein for IgM detection and RBD/S1 protein for IgG detection processes up to 80 samples/hour. In this work, the performance of IgM and IgG tests on the eCl system was evaluated in comparison with the current diagnostic routine method.

Materials and Methods

Performance was rated with 185 anonymized positive for SARS-CoV-2 IgG (with Screen Test COVID-19 IgM/IgG) serum samples collected from patients >15 days post SARS-CoV-2 molecular positivity and 284 negative samples from the preCOVID-19 era. Samples processed with eCL system were compared with SARS-CoV-2 IgM and SARS-CoV-2 S1 / S2 IgG tests on Liason® (DiaSorin, Saluggia, Vercelli).

Results

Overall, 185 and 284 SARS-CoV-2 positive and negative samples, respectively, were included in the study. Concerning IgG detection, eCL showed a sensitivity of 95.4% (167/175; CI 91-98; 1 failed measurement) and a specificity of 100% (282/282 CI 99-100). The positive predictive value (PPV) and negative predictive value (NPV) for IgG were 100% and 99.3% respectively. The eCL sensitivity in IgM detection was 65.5% (74/113; CI 56-74; 1 failed), and the specificity was 100% (276/276 CI 99-100; 3 failed). The PPV was 91.3 % (74/81 CI 84-96), whereas NPV was 100 % (276/276 CI 99-100).

Discussion and Conclusions

The study highlights high sensitivity and specificity rate of eCL ECLIA system for the detection of SARS-CoV-2 IgG comparable to the performance of other CLIA/ECLIA methods for the S protein target. Despite the low sensitivity of eCL IgM detection, the high variability of concentration during disease progress, makes poor significance in IgM evaluation. Rather, the high sensitivity in detecting IgG > 15 days after RT-PCR positivity and the high throughput may candidate eCL at routine diagnostic employment for retrospective screening of asymptomatic individuals or to evaluate the rate of immunized population. Since eCL IgG target was RBD/S1 protein, this system may be useful to monitor population IgG persistence and establish how long immunity might last.

50 - Ex vivo efficacy of currently licensed anti-SARS-CoV-2 monoclonal antibodies

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BACKGROUND. Two monoclonal antibody (mAb) cocktails, namely LY-CoV555/LY-CoV016 by Eli-Lilly (LYC) and REGN-COV2 by Regeneron (REG) have received emergency use authorization from the FDA for early treatment of mild to moderate COVID-19 in patients at high risk for progressing to severe COVID-19. The rapid emergence of SARS-CoV-2 variants is challenging the efficacy of mAbs designed based on the formerly prevalent B.1 lineage. The aim of this work was to test the efficacy of LYC and REG treatment in a small population of infected patients from clinical practice.

MATERIALS AND METHODS. Of 19 patients studied (9 males, mean (SD) age 62.4 ± 16.7 years), one was asymptomatic while the others developed mild symptoms such as cough (n=13), headache (n=10), fever (n=9), dysgeusia (n=3), dyspnoea or gastrointestinal symptoms (n=2). Two patients had received one and two SARS-CoV-2 mRNA vaccine doses. Patients were randomly treated with LYC (n=10) or REG (n=9) 2.9 ± 1.6 days from diagnosis. Sera were collected 1 hour before (baseline) and 1 hour post mAbs infusion. NtAb titres were determined in a live virus microneutralization assay performed in VERO E6 cell line using a quantitative readout based on cell viability. NtAb titres were defined as the reciprocal value of the sample dilution that showed a 50% protection of virus-induced cytopathic effect (ID_{50}) and determined against the wild type lineage and the paired isolate from each patient.

RESULTS. None of the patients required mechanical ventilation or hospitalisation. Seventeen and 2 patients harboured the alpha and gamma virus, respectively. All patients but the one completing two-dose vaccination (ID_{50} = 70) were negative for NtAb at baseline. The median [IQR] post-infusion NtAb titres were significantly higher ($p<0.001$) in REG *vs* LYC recipients against the wild type virus (20,820 [17,388-27,651] for REG *vs* 6,792 [4,736-7,777] for LYC) and even more against paired individual variants (117,453 [51,200-128,000] for REG *vs* 9,512 [4,878-17037] for LYC). However, the time from diagnosis to SARS-CoV-2 RNA negativization was not significantly different ($p=0.182$) with the two cocktails (17 vs 13 days; LYC vs REG) and was not correlated with NtAb titres both for wild type and for paired individual variant. Overall, the neutralization capacity was higher to the patient paired virus than to the wild type virus (18,558 [7,620-117,453] vs. 8,931 [6,461-20,820], $p=0.001$) but titres were highly correlated (rho = 0.728, $p<0.001$). One notable exception were the two gamma variants which were not neutralized by the LYC cocktail (Figure 1).

CONCLUSIONS. Both LYC and REG infusion achieve high virus neutralization capacity in vivo against currently prevalent variants, however the gamma lineage appears to be resistant to LYC.

56 - Combined host- and pathogen-directed therapy for the treatment of Mycobacterium abscessus infection.

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Introduction. *Mycobacterium abscessus* (Mab) is the etiological agent of severe pulmonary infections in vulnerable patients, such as those with Cystic Fibrosis (CF), where it represents a relevant cause of morbidity and mortality. Treatment of pulmonary infections caused by Mab remains extremely difficult as this species is resistant to most classes of antibiotics, including macrolides, aminoglycosides, rifamycins, tetracyclines, and β-lactams. In this study, we have analysed the therapeutic value of the combined treatment with apoptotic body like liposomes (ABL) loaded with phosphatydilinositol 5-phosphate (ABL/PI5P) and the antibiotic amikacin, both *in vitro* and *in vivo* models of Mab infections, as a model of combined host- and pathogen- directed therapy.

Materials and Methods. Differentiated THP-1 cells (dTHP-1), used as a model of human macrophages, and primary macrophages derived from CF patients, were treated or not with a Cystic Fibrosis Transmembrane conductance Regulator channel (CFTR) inhibitor, and *in vitro* infected with Mab. Cells were then stimulated with ABL/PI5P and/or amikacin, and treatment efficacy was evaluated in terms of extracellular and intracellular mycobacterial killing. The efficacy of the ABL/PI5P-amikacin combination therapy was also evaluated in wildtype and CFTR knockout mice chronically infected with Mab, in terms of pulmonary bacterial burden, leukocyte recruitment, cytokine production and kidney and liver toxicity.

Results. Results show that *in vitro* stimulation with ABL/PI5P and amikacin of dTHP-1 and CF macrophages, treated or not with inhibitor of CFTR, may significantly target extracellular and intracellular bacilli. Notably, the combined amikacin and APL/PI5P treatment promotes a significant higher reduction of intracellular Mab replication index when compared to single treatments. *In vivo* results show that single treatments induced about 10fold reduction of pulmonary mycobacterial burden, which was further improved to 100fold following combination therapy. Moreover, such a strong decrease of mycobacterial burden, observed after combination therapy, was associated with a highly significant reduction of inflammatory response, as analysed in terms of pulmonary differential leukocyte counts and IL-1beta, TNF-alfa, KC, and IFN-gamma production.

Discussion and Conclusions. These results support the therapeutic value of a combined host- and pathogen-directed therapy as a promising approach, alternative to single treatments, to simultaneously target intracellular and extracellular bacteria, and to reduce immunopathogenic response, for a better clinical management of patients infected with MDR pathogens, such as Mab.

57 - Fighting MDR-Klebsiella pneumoniae infections by a combined host- and pathogen-directed therapeutic approach.

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Introduction. *Klebsiella pneumoniae* (KP) is a opportunistic pathogen very difficult to treat mainly due to its high propensity to acquire complex resistance traits. Notably, multidrug resistance (MDR)–KP infections are responsible for 40-50% of mortality among hospitalized and immunocompromised patients. Although treatments with new drugs or with combined antibiotic therapies have some degree of success, there is still the urgency to investigate and develop an efficient approach against MDR-KP infections. In this study we have evaluated, in an *in vitro* model of human macrophages, the efficacy of a combined treatment consisting of apoptotic body like liposomes loaded with phosphatidylinositol 5-phosphate (ABL/PI5P), and phiBO1E, a lytic phage specific for the major high-risk clone of KPC positive MDR-KP.

Materials and Methods. Differentiated THP-1 cells, used as a model of human macrophages, were *in vitro* infected with the KKBO-1 strain, a representative of CC258 clade II, and then stimulated with ABL/PI5P and/or treated with phiBO1E. The therapeutic value of single and combined treatments was evaluated in terms of extracellular and intracellular bacterial load and pro- and anti-inflammatory cytokine secretion.

Results. Results show that ABL/PI5P did not affect in a direct manner KKBO-1 viability, being able to reduce only the intracellular KKBO-1 bacterial load. As expected, phiBO1E stimulation was effective mainly on reducing extracellular bacilli. Importantly, the combination of both treatments resulted in a simultaneous reduction of intracellular and extracellular bacilli. Moreover, the combined treatment of KKBO-1 infected cells reduced pro-inflammatory TNF-alfa and IL-1beta cytokines and increase anti-inflammatory TGF- beta cytokine production.

Discussion and Conclusions. Altogether our data support the therapeutic value of a combined host- and pathogen- directed therapy as a promising approach, alternative to single treatments, to simultaneously target intracellular and extracellular pathogens and improve the clinical management of patients infected with MDR pathogens, such as KP.

102 - Kinetics of neutralizing antibodies response to Comirnaty (BNT162b2) vaccine in healthcare workers

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Introduction: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which causes the disease COVID-19, is having a serious worldwide impact on human health. At the end of December 2020, few days after the approval of Italian Medicines Agency, Italy started its immunization campaign with a COVID-19 mRNA vaccine (Comirnaty-BNT162b2 Pfizer-BioNTech) on Health care workers (HCWs) that are at high-risk to acquire SARS-CoV-2 infection from patients or other fellow HCWs.

Materials and Methods: A total of 76 HCWs at the A.O.U.P. "P. Giaccone" Hospital (Palermo, Italy), vaccinated with BNT162b2 from 28 December to 16 February were enrolled in this study to investigate the antibodies response against SARS-CoV-2 vaccine, the kinetics and the persistence of anti-S1/S2 IgGs and neutralizing antibodies (Nt-Abs), during a four-month monitoring following the first dose of vaccine.

Results: Temporal analysis of SARS-CoV-2 Abs titre kinetics showed three different stages in Abs response, with an initial slow growth in the IgG and Nt-Abs titres during the first four weeks after the first dose of vaccine, followed by an antibodies peak around 35 days after first dose (i.e. 14 days after the second dose) and by a last stage (42 to 120 days after the first dose) with steady decrease of IgG-Abs and only a slight decrease of Nt-Abs which are then maintained at a stable titre over the time. When the study population was divided by sex and age higher levels of IgG- and Nt-Abs response were observed in females and in subjects aged 26 to 49 years. A statistically significant correlation was observed between anti-S1/S2 IgG- and Nt-Abs in the first stage of SARS-CoV-2 Abs kinetics, indicating that IgG Abs could provide indirect information on Nt-Ab titre at this stage, while the steady decrease of SARS-CoV-2 IgG-Abs titre observed in the third phase of the kinetics was not correlated to a proportional reduction of Nt-Abs, suggesting that Nt-Abs could maintain a stable titre over the time despite declining IgG Abs titre.

Discussion: The persistence over the time of specific Nt-Abs to SARS-CoV-2 Spike protein is fundamental for the protection from infection by blocking viral entry into host cells and may be crucial to provide durable humoral immunity. Care should be taken not to use specific IgG levels as a proxy of neutralizing humoral immunity if their kinetics has not been compared to Nt-Abs levels assessed through a neutralization test.

63 - Persistence of Neutralizing Antibodies to SARS-CoV-2 in First Wave Infected Individuals at Ten Months Post-Infection: the UnIRSA Cohort Study

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Abstract

Longitudinal mapping of antibody-based SARS-CoV-2 immunity is critical for public health control of the pandemic and vaccine development. Using sequential serum samples collected from a cohort of 100 COVID-19 recovered individuals from northern Italy—mostly with mild disease—at 2 (M2) and 10 (M10) months after their first positive PCR test, we show that 93% of them seroconverted at M2, with a geometric mean (GeoMean) ID50 neutralization titer of 387.9. Among the 35 unvaccinated subjects retested at M10, 7 resulted seronegative, with an 80% drop in seropositivity, while 28 showed decreased anti-RBD and anti-spike IgG titers, with a GeoMean ID50 neutralization titer dropping to 163.5. As an ID50 > 100 is known to confer protection from SARS-CoV-2 re-infection, our data show that the neutralizing activity elicited by the natural infection has lasted for at least 10 months in a large fraction of subjects.

130 - Evidence of SARS-Cov-2-specific memory B cells six months after vaccination with BNT162b2 mRNA vaccine

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Introduction. SARS-CoV-2 mRNA vaccines have demonstrated high efficacy and immunogenicity, but limited information is currently available on memory B cells generation and long-term persistence.

Materials and Methods. Here, we investigated Spike-specific memory B cells and humoral responses in 145 subjects, up to six months after the BNT162b2 vaccine (Comirnaty) administration.

Results. Spike-specific antibody titers peaked 7 days after the second dose and significant titers and neutralizing activity were still observed after six months, despite a progressive decline over time. Concomitant to antibody reduction, Spike-specific memory B cells, mostly IgG class-switched, increased in blood of vaccinees and persisted six months after vaccination. Following *in vitro* restimulation, circulating memory B cells reactivated and produced Spike-specific antibodies. A high frequency of Spike-specific IgG^+ plasmablasts, identified by computational analysis 7 days after boost, positively correlated with the generation of IgG^+ memory B cells at six months.

Discussion and Conclusions. These data demonstrate that mRNA BNT162b2 vaccine elicits strong B cell immunity with Spike-specific memory B cells that still persist six months after vaccination, playing a crucial role for rapid response to SARS-CoV-2 virus encounter.

125 - Short or Long Interval between Priming and Boosting: Does It Impact on the Vaccine Immunogenicity?

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Introduction. Characterizing the impact of the vaccination schedule on the induction of B and T cell immune responses is critical for improving vaccine immunogenicity.
Materials and Methods. Here we compare the effect of a short (4 weeks) or a long (18 weeks) interval between priming and boosting in mice, using a model vaccine formulation based on the chimeric tuberculosis vaccine antigen H56 combined with alum.
Results. While no significant difference was observed in serum antigen-specific IgG response and the induction of antigen-specific T follicular helper cells into draining lymph nodes after the two immunization schedules, a longer interval between priming and boosting elicited a higher number of germinal center-B cells and H56-specific antibody-secreting cells and modulated the effector function of reactivated CD4+ T cells.
Discussion and Conclusions. These data show that the scheduling of the booster immunization could affect the immune response elicited by vaccination modulating and improving the immunogenicity of the vaccine.

103 - Improved xenophagy through recombinant BCG as a strategy for new generation vaccine against tuberculosis.

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INTRODUCTION

Many efforts are carried out to develop a new generation of vaccines against tuberculosis (TB) with higher efficacy than that afforded by Bacillus Calmette-Guerin (BCG). One hypothesis claims that the ability of BCG to partly resist intracellular killing may hamper antigen presentation, limiting its immunogenicity. With this purpose, our group attempted to develop BCG strains capable of triggering a more efficient immune response by enhancing phagosome maturation through xenophagy activation.

MATHERIALS AND METHODS

We generated four plasmids expressing the Mpt64 mycobacterial antigen fused either with murine ubiquitin or murine LC3, two proteins involved in xenophagy pathway. The plasmids were electroporated in BCG and stable protein expression was verified through SDS-PAGE and immunoblot. Intracellular viability of these BCG strains was tested in vitro infection assays on murine macrophage cell line J774.A1 by Colony Forming Units (CFU) counting at 4 and 7 days post infection (p.i.). Finally, we measured the protection induced by PRO-A strains in vivo murine infection model through CFU count on lungs and spleens homogenate at 28 and 60 days p.i., and through granuloma counts.

RESULTS

Immunoblot showed a stable expression of proteins at the expected molecular weights. In vitro infection assays, recombinant BCG PROA-A strains exhibited a slightly minor persistence capacity, compared with the parental BCG Pasteur strain at 4 and 7 days p.i., even though strain expressing Mpt64-LC3 showed an increase at 7 days post infection. As for in vivo protection experiments, mice vaccinated with BCG Mpt64-Ub and BCG Mpt64-LC3 afforded superior level of protection as indicated by the lower CFU count at day 28 p.i. in the lungs. Moreover, mice vaccinated with these pro-A strains showed reduced number of tubercles and of reduced size compared to BCG control strain; however at day 60 p.i. there are no differences between vaccinated groups; in all cases there are always remarkable differences respect with unvaccinated. At day 60 p.i. this difference is less evident.

DISCUSSION

In this study, we showed that enhancing phagosome maturation through xenophagy pathway may promote more efficient immunogenicity of BCG-based vaccines. The use of this BCG enhancement strategy together with others strategies already used successfully, such as the overexpression of Mycobacterium tuberculosis antigen, could lead to the development of a new vaccine against TB.

31 - Quantification of SARS-CoV-2 neutralizing antibodies after vaccination in COVID-19 positive and negative Italian healthcare workers

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INTRODUCTION: The world is in the midst of a Coronavirus Disease 2019 (COVID-19) pandemic. Vaccines are a critical new tool in the battle against COVID-19. Up to date, a total of 940 million doses of COVID-19 vaccines have been administered in the world. The degree of immunity conferred by natural infection and/or vaccination are still being investigated. The aim of the study was to detect the presence and magnitude of neutralizing antibodies (NAbs) against SARS-CoV-2 raised after vaccination with the mRNABNTA62b2 Cominarty vaccine (Pfizer-BioNTech).
MATERIALS AND METHODS: Microneutralization assay was employed to detect neutralizing antibodies on human serum samples taken from vaccinated healthcare workers with (group 1) and without (group 2) a previously laboratory confirmed COVID-19 diagnosis at the Istituto Clinico Città Studi (ICCS) hospital in Milan (Lombardy, Italy). Serum samples were collected from the first group before (T0) and after the two doses of (T1 and T2) and from the second group, twenty days after the first dose (T1) and two weeks after the second dose (T2). The 50% endpoint titre was determined.
RESULTS: A total of 8 subjects for group 1 and 15 subjects for group 2 were enrolled. Median age of subjects was 46.0 years (range: 26-52) for group 1 and 50.9 (range: 26-65) for group 2. Median neutralizing antibody titres were 1:12.8 before vaccination (group 1, T0), 1:664.0 (group 1, T1) and 1:2.7 (group 2, T1) after first dose of vaccination and 1:724.0 (group 1, T2) and 1:45.2 (group 2, T2) after second dose of vaccination.
DISCUSSION AND CONCLUSIONS: All subjects developed neutralizing antibodies against SARS-CoV-2 after administration of the vaccine. Subjects who were previously infected with SARS-CoV-2 showed much higher SARS-CoV-2 50% endpoint titre after administration of both the first and second vaccination dose than subjects who were never infected before vaccination. The neutralizing antibody levels increased after administration of the second dose both groups. Not many literatures about the human immunity against SARS-CoV-2 after vaccination is still available. This study provides useful information about natural immunity and acquired immunity after vaccination that could be employed in extensive serological surveys, vaccination plans and new therapeutic approaches.

44 - Decreased neutralization of the B.1.525 (Nigerian) SARS-CoV-2 variant by sera of previously infected and uninfected vaccinated individuals.

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INTRODUCTION. Emergent SARS-CoV-2 variants of concern (VOC) and variants of interest (VOI) are challenging the immune protection resulting from natural and artificial immunization with the original B.1 virus variant. The VOI B.1.525 combines relevant spike mutations detected in several VOC, yet its potential for vaccine escape has been poorly investigated. In this study we determined serum neutralizing antibody (NtAb) response to B.1.525, as well as to other viral variants, in a cohort of health care workers (HCWs) including both previously infected and uninfected individuals, all vaccinated with two doses of the BNT162b2 mRNA vaccine.
MATERIALS AND METHODS. 15 HCWs (median age [IQR] 38 [31-52] years, 8 females) infected during the first wave of the pandemic tested at baseline ($T0_{inf}$) and 17±6 days after receiving the second vaccine dose ($T2_{inf}$) were studied. The control group included 15 uninfected HCWs (median age [IQR] 38 [29-59] years, 11 females) tested 18±4 days after the second dose vaccination ($T2_{uninf}$). NtAb titer to live virus variants belonging to lineage B.1, P.1, B.1.1.7 and B.1.525 was determined by a microneutralization assay performed in VERO E6 cells using as readout the quantification of cell viability (Promega). The NtAb titer was defined as the reciprocal value of the sample dilution that showed a 50% protection of virus-induced cytopathic effect (ID_{50}). The anti-spike protein Ab was quantified by SARS-CoV-2 IgG II Quant assay (Abbott).
RESULTS. In previously infected HCWs, NtAb titres to all viral variants significantly increased after vaccination (mean $T2_{inf}/T0_{inf}$ ratio 119±66; $p<0.001$). Also, the NtAb titer after vaccination was higher in the previously infected compared with the uninfected group (mean $T2_{inf}/T2_{uninf}$ ratio 6±2; $p<0.001$). Overall, NtAb titres to the B.1.525 strain (63 [7-323]) correlated well with those to B.1 (133 [9-456], P.1 (148 [46-988]) and B.1.1.7 (87 [5-681]) ($p<0.001$). NtAb titres to B.1.525 were significantly lower with respect to those obtained for each variant ($p<0.001$). Anti-spike protein Abs also correlated with NtAb titres to B.1 (rho = 0.934), P.1 (rho = 0.914), B.1.1.7 (rho = 0.913) and B.1.525 (rho = 0.918) viruses ($p<0.001$). Also, a significant increase was observed when comparing the anti-spike Ab median titres at $T2_{inf}$ and at $T0_{inf}$ (27763 [18282-46108] vs. 1.7 [0.5-4.4]; p=0.001).
DISCUSSIONS AND CONCLUSIONS. NtAb elicited by natural or artificial immunisation with the original B.1 lineage cross-neutralize multiple viral variants. However, neutralization of B.1.525 is significantly reduced with respect to other variants. Indeed, NtAb titres could be ranked with the definite order P.1>B.1=B.1.1.7>B.1.525. Despite reassuring *in vitro* data, *in vivo* protection remains to be confirmed.

03 Diagnostica microbiologica

138 - Phylogenetic analysis of Staphylococcus microti associated with sub-clinical mastitis in Bubalus bubalis

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Introduction Water buffalo (*Bubalus bubalis*) is of great economic importance as a provider of milk in Campania Region (Italy) that represents an indispensable source for the typical Mozzarella cheese production. The "Mozzarella di Bufala Campana" is the only mozzarella which obtained and retain the PDO (Protected Designation of Origin) European designation. Mastitis, a breast infection, is one of the most expensive diseases affecting the dairy industry and particularly the sub-clinical mastitis are a big problem because often not diagnosed. **Materials and Methods** The research was conducted in a buffalo farm of Campania Region and 160 milk samples were checked for somatic cell count (SCC) as well as for microbiological culture using different media. The isolated bacteria were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). As a complementary and confirmation approach, 16S rRNA gene sequence analysis was performed. **Results** The testing of SCC showed values >200,000 cells/mL in 14 milk samples. The microbiological cultures resulted positive to the growth of 45 strains of *Staphylococcus microti*. These milk samples showed the growth of colonies with phenotypic characteristics of the genus *Staphylococcus*. The identification by MALDI-TOF-MS with a log(score) ≥2.0 indicated that identification was reliable at the species level as *Staphylococcus microti*. The obtained mass spectra were statistically compared by Principal Component Analysis (PCA) that revealed three different clusters. Three *S. microti* strains which were associated with high number of SCC, indicative of sub-clinical mastitis, were further processed by the sequencing of 16S rRNA gene by using genetic analyzer (Life T 3730 DNA Analyzer). **Discussion and Conclusions** Our results indicate that the bacterial identification of *Staphylococcus microti*, a novel staphylococcal species, based on MALDI-TOF MS analyses was perfectly confirmed by the classification method based on complete 16S rRNA gene sequence analyses. In addition, MALDI-TOF MS analysis has further improvement potential respect to 16S rRNA gene analysis, so it could be a faster and cheaper method of identification.

68 - Spread of respiratory viruses during the COVID-19 pandemic among children in the North-East of Italy

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Introduction: The COVID-19 pandemic has led to a profound change in the behavioural habits of the population. The social distancing measures caused a remodelling of the epidemiology of respiratory viruses during the winter season, however, data from children are still limited. This observational study aims to analyse the impact of restriction measures against SARS-CoV-2 on the shaping of the circulation of common winter respiratory pathogens in the paediatric population of the North-East of Italy. **Materials and Methods:** From September 2020 to March 2021, a total of 1227 nasopharyngeal swabs from symptomatic paediatric patients (0-17 years old) afferent to the Maternal and Child Hospital IRCCS Burlo Garofolo (Trieste, Italy) were tested. Respiratory Flow Chip assay (Vitro, Sevilla, Spain), including SARS-CoV-2, Influenza A and B, Adenovirus, other Coronaviruses, Parainfluenza Virus 1–4, Enteroviruses, Bocavirus, Metapneumovirus, Respiratory Syncytial Virus, Rhinoviruses, *Bordetella pertussis*, *Bordetella parapertussis* and *Mycoplasma pneumoniae*, was used for the analyses. To relate virus positivity with the clinic characteristics of the subjects enrolled, bivariate logistic models were estimated. **Results:** SARS-CoV-2 was detected in 5% of the children, showing a peak of prevalence in those under the age of two. Fever resulted to be a risk factor for COVID-19 infection (OR=2.03, p=0.03). Among other microorganisms, Rhinovirus was detected in the 41% of the subjects, cough and rhinitis were risk factors (respectively OR=2.17, p<0.001 and OR=2.86, p<0.001). Other Coronaviruses were found in 11% of children, most frequently in those under the age of 5, and were associated to pharyngodynia (OR=2.89, p<0.001). Adenovirus, observed in 12% of subjects, showed to have fever as risk factor (OR=5.66, p<0.001). Bocavirus was detected in 3,2% of children between 2 and 5 years old. **Discussion and Conclusions:** The social isolation measures adopted during the COVID-19 pandemic led to the reduction of the circulation of some common respiratory pathogens such as RSV and Influenza in the paediatric population. However, this study highlights the need for continuing surveillance, including the impact of the new variants of SARS-CoV-2 on the diffusion of the other viruses.

139 - Whole-genome sequencing based identification of a prolonged Central Venous Catheter-Related Bacteremia by Gordonia terrae in a Pediatric Oncology Patient

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Introduction

Gordonia terrae is a, thin, beaded nocardioform Gram-positive organism ubiquitous in the environment, being widely distributed in soil and water. Nevertheless, it has been occasionally recognized as cause of nosocomial infections in immunocompromised patients. To date, only a limited number of reports have been documented, exclusively involving adult patients. Here, we report a case of CR-BSI caused by *G. terrae* in a pediatric oncology patient, identified using whole-genome sequencing (WGS).

Materials and Methods

G. terrae, on 21 October 2020, was cultured from Chocolate Agar plate (Liofilchem, Roseto degli Abruzzi, Italy). Species-level identification was carried out by MALDI-TOF MS (Vitek MS; bioMérieux). The isolate was subjected to WGS using the Oxford Nanopore MinION platform (Oxford Nanopore Technologies, UK). Assembly of raw reads was performed using Flye, and the Type Strain Genome Server (TYGS) was used for bacterial identification. The presence of resistance determinants was inspected through ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/). Antimicrobial susceptibility testing was assessed using a commercial plate (Sensititre™ RAPMYCO2 Susceptibility Testing Plate), according to the CLSI guidelines for aerobic actinomycetes.

Results

In October 2020, during hospitalization for an immunotherapy course, the patient became febrile. Blood cultures were performed and, after 24-72h, the growth of a Gram-positive coryneform organism was recorded. Attempts to achieve bacterial identification by MALDI-TOF MS were unsuccessful. Long-reads sequencing resulted in a draft assembly including 2 contigs (total length: 5660173; N50: 5579821; smallest contig: 80352), and WGS-based identification as *G. terrae* has been then achieved. No resistance determinants were detected. Consistently, the isolate was consistently susceptible to all tested antibiotics. An empirical antimicrobial therapy with ampicillin (1000mg q.i.d. for 7 days) was started and was then modified with vancomycin continuous infusion (40mg/kg/day) due to persistently positive blood cultures. After removal of the central venous catheter, a therapy with ceftriaxone (80mg/kg/day) was started and no further positive blood cultures were identified.

Discussion and Conclusions

Although infections by *G. terrae* are rare, either because this genus if often not recognized as clinically significant or the isolates is misidentificated by conventional identification systems, the role of this organisms as cause of medical device related infections in immunocompromised pediatric patients should deserve major attention. In this context, WGS proved to be a valuable tool to be implemented in the diagnostic routine for a reliable identification of fastidious bacteria or slow-growing organisms.

49 - Fast-track diagnostics for Gram-negative bloodstream infections: MALDI-TOF and lateral flow immunoassays directly from blood culture bottles.

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Introduction: In the era of multi drug resistant Gram-negative infections, integration of rapid diagnostics is the key that supports timely transition to effective therapy, including the appropriate use of the new combinations beta-lactam-beta-lactamase inhibitor.
This study aims at presenting a reliable fast-track diagnostics for the rapid identification of Gram-negative pathogens, and detection of CTX-M ESBL- (CTX-M-p) and carbapenemase-producers (CA-p) *Enterobacterales* (EB) directly from positive blood cultures.
Material and methods: This two-year study (2019-2020) included non-duplicate positive BCs with Gram-negative rods at microscope observation. Fast-track diagnostics consisted of rapid microbial identification by MALDI-TOF MS directly from BC fluid, and rapid detection of main carbepenemases and CTX-M-ESBL production on samples tested positive to *Klebsiella pneumoniae* or *Escherichia coli*, using rapid lateral flow immunoassays (LFIAs) NG-test Carba 5 and NG-test CTX-M MULTI. Bacterial identification was considered reliable with a score of >1.80.
Rapid diagnostics results were then compared to those obtained with conventional diagnostic routine.
Results: Over the study period a total of 1063 BCs were processed. Among these, 835 (78.5%) and 278 (26.1%) were positive for EB and Gram-negative non-fermenters species, respectively. In detail, *E.coli* was the most observed EB specie (n=378, 35.6%) followed by *K.pneumoniae* (n=223, 21%), *Pseudomonas aeruginosa* (n=114, 10.7%), *Enterobacter spp*. (n=98, 9.2%), and *Acinetobacter baumanni* (n=37, 3.5%). Overall, direct MALDI-TOF MS analysis provided reliable identification of bacterial species and in agreement with the conventional MALDI-TOF identification in 897 out of 1008 (89%) monomicrobial BCs. NG-Test Carba 5 assay identified 64 (28.7%) CA-p *K.pneumoniae* (KPC n=64, NDM n=1, VIM n=1, OXA-48 n=1, KPC/VIM n=1) and 5 (1.3%) CA-p *E.coli* (VIM n=4, KPC n=1). The assay showed discordant results with conventional routine only in two cases, regarding ceftazidime-avibactam resistant KPC-producing *K. pneumoniae* that were not identified as carbapenemase producers. NG-test CTX-M MULTI identified 87 (39%) CTX-M-p *K. pneumoniae* and 70 (18.5%) CTX-M-p *E.coli*. The assay identified 588 out of 601 (97.8%) of ESBL producers and showed no false positive results.
Discussion and Conclusions: There was considerable agreement between the results provided by our fast-track workflow and conventional diagnostic methods. Despite the requirement for the later to confirm fast-track workflow results and to obtain a detailed pattern of antimicrobials activity, rapid tests for the detection of the more common resistance mechanisms are an invaluable tool in setting timely antimicrobial stewardship interventions, thus potentially improving clinical outcomes.

93 - Rapid presumptive diagnosis of Acinetobacter baumanii pneumonia in respiratory specimens through cross-reactivity with Pneumocystis jirovecii direct immunofluorescence assays

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INTRODUCTION. *Acinetobacter baumannii* pneumonia is a leading cause of morbidity and mortality in intensive care units, especially in patients undergoing mechanical ventilation (ventilator-associated pneumonia, VAP). One of the main reasons is that this Gram-negative rod is usually resistant to several antimicrobials through a wide array of molecular mechanisms, making it a difficult target to challenge and eliminate. Extremely worrying is the wide distribution of carbapenem resistant strains of *A. baumannii*, which usually forces to the use of drugs of last resort. This highlights the importance of introducing means of rapid diagnosis, in order to hamper the bacterial growth in the context of the patient's respiratory tract as soon as possible by setting a more appropriate empiric therapy before the results of a standard cultural diagnosis with antimicrobial susceptibility testing, which may need several hours if not days to be performed. A diagnostic tool able to reduce the time to treatment with appropriate antibiotics would perfectly fit in the strategies of antimicrobial stewardship. **MATERIALS AND METHODS.** We here describe a novel instrument, which appears not to be described in the available literature, that could be useful for this purpose. **RESULTS.** We found that *A. baumannii* cross-reacts with monoclonal antibodies used for direct immunofluorescence assays (DFA) to detect *Pneumocystis jirovecii* in profound respiratory samples, becoming visible as bright green coccobacillary rods. In particular, the cross-reaction appears to occur at the membrane level. This was confirmed by fixing colonies of *A. baumannii* on immunofluorescence slides and treating them with the same protocol. The same result was not obtained with colonies of the other main bacteria responsible of VAP, i.e. *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae,* which could mean that this cross reaction specifically regards Acinetobacter. **DISCUSSION AND CONCLUSIONS.** Thus, a DFA for *P. jirovecii*, usually performed as an urgency in many laboratories, could be useful to suggest as soon as possible to the clinicians an infection sustained by *A. baumannii* in a VAP setting, alongside the diagnosis of an eventual *P. jirovecii* pneumonia. Future expansions of this research could regard the definition of the cross-reactive domains and the clinical impact of this strategy.

19 - Comparison of different diagnostic procedures for Pneumocystis jirovecii detection

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Introduction: the present study aimed at comparing the performance of microscopic, end-point and quantitative real-time PCR (qPCR), and (1-3) Beta-D-glucan (BDG) assay examinations of bronchoalveolar-lavage (BAL) fluids from patients with suspected *Pneumocystis jirovecii* pneumonia.

Matherials and Methods: Sixteen BAL from patients selected on the basis of strict inclusion criteria and four BAL from control patients were examined with Grocott-Gomori's methenamine silver-staining (GMS) microscopy, end-point PCR and qPCR, and BDG assay.

Results: Seven samples (43.8%) out of 16 had a positive GMS microscopy; end-point PCR and qPCR detected *P. jirovecii* DNA in 8 (50%) and 9 samples (56.3%), respectively; BDG results were positive for 15 samples (93.8%). All positive GMS samples had concordant PCRs and BDG results. An additional sample was detected by end-point PCR as weakly positive and by qPCR. Another sample was detected only by qPCR. As BDG is present in the cell-wall of many fungi, BDG results were positive in almost 94% of the selected patients. Negative control patients were all negative for microscopy, end-point PCR, qPCR, and BDG assay.

Discussion and Conclusions: Upon careful selection of patients, molecular methods can provide reliable and fast diagnosis of *P. jirovecii* infection. DNA-based techniques are easier to perform and interpret and more sensitive than microscopy. BAL BDG assay proved to be useful in ruling out the diagnosis of *Pneumocystis jirovecii* pneumonia due to its highly negative predictive value.

48 - Detection of Mycoplasma contamination in cell cultures

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1. Introduction

Mycoplasma (*Mp*) are parasitic bacteria that adhere to cellular surfaces, naturally resistant to many antibiotics and extremely small. They are often found as contaminants in cultured cells, where they go unnoticed. They may be present in viral stocks because they are present in supernatants of cells where cultured viruses are released. The best way to keep laboratories free of *Mp* is to discard infected cultures, but, as judged by the very common finding of *Mp* -contaminated cultures in many laboratories, this is not done as often as it should be. A possible reason is that most procedures recommended take as long as performing a simple experiment and many laboratories delay testing to save money and time. Indeed, many methods exist to detect *Mp* infection of cell lines, but they take at least a couple of hours of hands-on work, if not more. Here we describe our experience with a procedure to screen viral stocks and tissue cultures for *Mycoplasma* presence. and does not require experienced personnel or expensive equipment. It only requires minutes of hands-on work and, for this, it may be useful for weekly screening of cultures. It yields semiquantitative results in roughly 5 days.

2. Materials and Methods

Isolation of contaminating *Mp* on horse blood Columbia agar (HBCA) was directly performed from exhausted tissue culture supernatants. Species identification was performed by sequencing the PCR-amplified 356-bp stretch of the 16s gene.

3. Results

We tested our method on a number of cell culture supernatants from 11 different laboratories and on viral stocks and found that most labs, if not all, had contaminated cultures. A predominant species was found in single laboratories. The most frequent species was *M. arginini*, followed by *M. hyorhinis*. We also tested viral stocks and, again, most yielded a patina of *Mp*, even after 1-2 years storage at -80°C.

4. Discussion and Conclusions

Because of its simplicity, the method described may be useful for detecting *Mp* in viral stocks and for frequent screening of cultures in research laboratories. Many laboratories are contaminated without knowing and there tends to be a specific strain that passes to different cell lines in the same laboratory.

118 - The infection rate of respiratory viruses in pandemic SARS-CoV2 period (October-2019-March 2021): an observational study

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Background: Concurrent circulation of SARS-CoV-2 and human common respiratory viruses may prove to be a challenge for health care providers, clinical microbiology and public health laboratories. The ability to differentiate the diseases caused by these viruses is essential for patient management and infection control, as well as public health surveillance because they can cause very similar symptoms, making clinical differentiation between them very difficult. In this study, we investigate the epidemiological, clinical and virological characteristics of patients with suspected respiratory pathogens in severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) pandemic period and clarify the actual infection rate of these viruses and possible correlations between respiratory viruses and SARS-CoV2. **Methods:** This was a retrospective study investigated on rate of infection of respiratory viruses in SARS-CoV2 epidemic situation in Microbiological unit of "Tor Vergata" University COVID-Hospital of Rome, from October 2019 to March 2021. The Film Array Respiratory Panel 2 (RP2) used for detection of respiratory viruses and SARS-CoV-2 real-time quantitative PCR for detection of SARS-CoV2 from March 2020 to January 2021. All data collected on database and used for statistical and epidemiological analyses. **Results:** A total of 1652 patients with suspicious of symptoms of respiratory infections were included in this study (162 patients were excluded for data missing), 1025 were men and 627 women. Frequently detected pathogens included SARS-CoV-2 (41,4%, 577/1397; from March 2020 to March 2021), Inf A-B (2,4%, 40/1652), HCOV (2,3% 38/1652) HSRV (1,5%, 25/1652), an inverse trend on the rate of positive cases considering common respiratory viruses. In fact, at the seasonal peak of October-March 2019-20, respiratory flu viruses are opposed to the absence of peaks in the period October-March 2020-2021. On the other hand, the epidemiological curve shows an increase and prevalence of positive cases of SARS-CoV-2, especially from October to March 20-21. Seven patterns of co-infection were evaluated in this surveillance research. It was found that 0,06% (4/577) of SARS-COV-2 positive cases had coinfections with other viruses. **Conclusion:** This study reveals the epidemiological features of common respiratory viruses and their clinical impact during the ongoing outbreak of SARS-CoV-2 in an epidemic area. Our results suggest how, in pandemic period, SARS-CoV2 had a preferential tropism in respiratory tract infection as a monovirus and not as a co-existing with other viruses. The possible causes were attributable either to the use of masks in Italy, or to particular respiratory receptors most available for these viruses or external and internal lifestyle factors.

143 - ANALYSIS OF HUMAN PAPILLOMAVIRUS (HPV) VIRAL LOAD IN WOMEN WITH CERVICAL DYSPLASIA

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Introduction

Persistent high-risk Human Papillomavirus (hrHPV) infection is the necessary cause of cervical cancer development. Cervical cancer is the fourth most frequent tumour among women with an estimated number of incident cases worldwide of 604,127 in 2020. The introduction of HPV testing in screening programs allowed to improve early prevention; however, new biomarkers are necessary to discriminate transient from persistent infections, such as HPV viral load estimation. This preliminary study aimed to analyse the prevalence and distribution of hrHPV genotypes in women with recent cervical dysplasia using two different sample collection methods and to evaluate the possible influence of hrHPV viral load in cervical cancer progression.

Materials and Methods

Physician-collected cervical specimens (L-Shaped Endo/Esocervical FloqSwab®, Copan) and vaginal self-samples (FloqSwab®, Copan) were obtained from 83 women referred to colposcopy for cervical dysplasia and resuspended in 20 ml and 5 ml of PreservCyt (Hologic), respectively. Nucleic acids were extracted by means of silica beads techniques and analysed using Oncopredict assay (Hiantis) to identify and quantify 12 hrHPV genotypes. Viral load was normalized respect to sample cellularity and expressed as Log10 of viral copies/10,000 cells. Cohen's kappa was used to evaluate results agreement.

Results

hrHPV positivity rate of 82% (68/83) was obtained from the analysis of cervical samples. HPV16 (39.4%) and HPV31 (14.9%) resulted the most prevalent genotypes. A good concordance (k=0.77) in high-risk HPV detection was found comparing cervical and vaginal self-samples results, even if multiple hrHPV infections occurred more frequently in vaginal specimens (28.9% and 34.9%, cervical vs vaginal sample, respectively). HPV16 Log10 median viral load were higher among cervical samples of women with CIN3 compared to CIN2 grade cervical dysplasia (4.18 vs 3.54). The same trend was also shown among vaginal self-samples (3.30 vs 2.74).

Discussion and Conclusions

These preliminary data demonstrated a good agreement from the comparison of results obtained from cervical and vaginal samples suggesting the possible use of self-collection in cervical cancer screening. Higher HPV16 viral load was observed among women with higher grade of cervical lesions, however results obtained from a larger population could clarify preliminary data obtained in this study.

132 - Evaluation of two extraction-free SARS-CoV-2 detection systems

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Introduction

Real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) analysis of nasopharyngeal swabs (NPS) represents the reference method for SARS-CoV-2 infection diagnosis. Several analytical platforms allow high-throughput testing but require relatively long turn-around times, due to the expensive RNA extraction step prior the PCR set-up and thermal cycling. The need of reducing time and cost of SARS-CoV-2 detection arose during pandemic waves, when large amount of samples needed to be rapidly tested and lack of reagents is a common issue. The aim of this study was to evaluate the performance of rapid extraction-free protocols using two commercial rRT-PCR assays.

Materials and Methods

Samples were anonymized residual NPS in UTM® medium (Copan, Italy): 139 were evaluated with Allplex™ SARS-CoV-2 (ALLCOV, Seegene Inc, South Korea), and 69 with Allplex™ SARS-CoV-2/FluA/FluB/RSV (ALLCOV-FLU). Different extraction methods were performed: i) conventional RNA extraction with the STARMag 96 X 4 Universal Cartridge Kit (Seegene Inc, South Korea) (ALLCOV and ALLCOV-FLU); ii) extraction-free by 1:4 sample dilution in RNase-free water (ALLCOV and ALLCOV-FLU); iii) extraction-free by 1:4 sample dilution in RNase-free water and heating at 98 °C for 3 minutes (ALLCOV). Samples were processed using a Hamilton Microlab NIMBUS automated extraction and PCR setup system.

Results

Conventional RNA extraction was used as reference method to compare results. Among the 96 SARS-CoV-2-positive NPS with ALLCOV, sensitivity of extraction-free with and without heating step was 57.2% (41 false negatives) and 73.9% (25 false negatives), respectively. Average cycle threshold (Ct) values of all viral targets were higher with extraction-free methods and average delta Ct was higher when heating step occurred. Among the 39 SARS-CoV-2-positive NPS with ALLCOV-FLU, sensitivity of extraction-free method was 82% (7 false negatives) with higher average Ct and delta Ct for all viral targets. False negatives results yielded by the extraction-free methods with both assays were observed in samples showing high Ct values.

Discussion and Conclusions

Skipping the conventional RNA extraction step proved to be a faster and cheaper approach to detect viral target in NPS maintaining a good sensitivity and the high throughput of the process. Heating step showed a greater loss of sensitivity, although both extraction-free methods showed loss of accuracy in samples with lower viral load. Overall, a good concordance with reference extraction method was observed, allowing a possible use of extraction-free methods for the diagnosis of SARS-CoV-2 and, if needed, of other viral pathogens.

136 - Antibody-functionalization of a Surface Acoustic Wave (SAW)-based biosensor for the rapid detection of Legionella pneumophila in water

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Introduction. Legionella spp. are hydro-dispersed bacteria, frequently associated with nosocomial infections, in particular in immunocompromised individuals. Due to aging populations, rising numbers of immunocompromised individuals and increased need for conditioned water due to climate change, such infections are constantly rising making mandatory the implementation of strategies for the control waterborne risk of *Legionella* infections. A Surface Acoustic Wave (SAW) biosensor comprises a Lithium Niobate substrate with interdigital transducers (IDTs) for SAW-excitation and microstructured reflectors for acoustic energy confinement. The adsorption of small amounts of an analyte on the sensor leads to a frequency shift of the acoustic cavity that can be measured. This system allows fast determinations, does not require expensive supportive equipment and selective coatings of the surface can be applied. Aim of the present work was the functionalization of a SAW-sensor with an antibody specific for the detection of *L. pneumophila* sg1 in a fluid.
Materials and Methods. The affinity of the polyclonal antibody Ab20527 was tested by ELISA assay against 3 environmental isolates (EI) of *L. pneumophila* sg1, 3 EI of *L. pneumophila* sg2-14, 1 EI of *L. longbeachea* and the ATCC strain 33152 (*L. pneumophila* sg1). The assay was performed on bacterial suspensions of 10^8 and 10^7 CFU/ml with the Ab20527 [1:25000], in triplicate. The sensor surface functionalization was a two-step process performed by pumping the solutions through the microfluidic layer coupled to the SAW chip. The first step was a treatment with a streptavidin-polyethylene-glycol (2 kDa)-thiol (sPEG) (NANOCS, New York, USA) linker; the second step was the loading of the biotinylated antibody Ab20527.
Results. ELISA assays using Ab20527 antibody showed a good sensitivity, detecting bacteria diluted up to 10^7 CFU/ml and showed high specificity when tested against other bacterial species (*P. aeruginosa, E. coli, S. epidermidis* and *E. faecium*). When functionalized with Ab20527, the SAW-sensor detected *L. pneumophila* in a dose-dependent manner with a lower limit-of-detection of 2.01E6 CFU/ml (5.51E3 CFU/chip) in tap water. No significant signal was detected when the antibody-functionalized SAW-sensor was incubated with other bacterial species (*E. co*li and *E. faecium*).
*Discussion and Conclusions***.** The SAW-sensor technology demonstrated the ability to detect *L. pneumophila* with high specificity whereas the sensitivity can be further improved by using an appropriate system of water concentration before the exposure to the sensor. (*This work was supported by "Regione Toscana" Bando FAR FAS 2014; Project "SENSOR").*

70 - Molecular epidemiology of sexually transmitted pathogens in a selected area of Campania region

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INTRODUCTION: Sexually transmitted infections (STIs) remain one of the major serious worldwide problem of public health that affect both women and men. In 2016, the World Health Organization reported an estimated 376 million infections of the four most common curable STIs (chlamydia, gonorrhoea, trichomoniasis and syphilis). The objective of this study was to provide an update information on the prevalence and co-infection of *Gardnerella vaginalis*, *Ureaplasma parvum, Ureaplasma urealyticum, Trichomonas vaginalis, Mycoplasma genitalium, Mycoplasma hominis,* by retrospectively analyzing a cohort of patients living in the metropolitan area around Naples, Campania region in Southern Italy. **MATERIALS AND METHODS:** To investigate the prevalence of sexually transmitted infections (STIs) in clinical specimens (vaginal/endocervical swabs and urines) collected from infertile asymptomatic female and male from 1st November 2018 to 31st December 2020 attending our institution, the University of Naples "Federico II" Hospital, we used a multiplex Real-Time PCR (mRT-PCR) assay. **RESULTS:** Of the 717 specimens collected, 302 (42.1%) resulted positive for at least one of the targets named above. The STI pathogens significantly affect female patients (52.3%) than male subjects (31.6%), with a risk 2.37 fold higher. Moreover, the age group 30-39 years analyzed, showed a higher risk of contracting STIs (O.R. 1.66). *G. vaginalis* and *U. parvum* represented the most common findings with a prevalence of 80.2% and 16.9% in vaginal/endocervical swabs and first-voided urines respectively. Prevalence of multiple infections was 18.1% and 8.2% in female and male patients, respectively; with a higher risk for female subjects (O.R. 2.36). The most frequent co-infection detected was "*G. vaginalis* & *U. parvum*" with 60% of prevalence. **DISCUSSION AND CONCLUSIONS:** Our epidemiological data clearly outline the profile most exposed to STIs: women aged between 30-39 years. These data highlight the need to implement a preventative screening strategies of sexually transmitted pathogens to reduce the complications on reproductive organs. Furthermore, the role of these sexually transmitted microorganisms will have to be further investigated to understand their real involvement not only in the clinical pathological pictures but also in the state of well-being of the reproductive system.

154 - A multidisciplinary COVID Diagnostic Center for a rapid diagnosis in asymptomatic patients in Genoa, Italy

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Introduction. Asymptomatic patients may play an important role in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission. Early detection of this patients becomes essential to stop the virus transmission. Since October 2020 ASL3 Sistema Sanitario Regione Liguria has placed many clinics in Genoa city (about 560,000 inhabitants) where asymptomatic people may undergo a free nasal swab for the rapid detection of SARS-CoV-2 antigen using first generation rapid assays. Among the first in Italy, in December 2020 we transformed one of these clinics into a multidisciplinary diagnostic center. This center is located in Villa Durazzo Bombrini in west of Genoa. It includes various professional figures and provides the patient with the entire way from anamnesis to confirmation molecular swab.

Materials and methods. About 120 people a day are tested. We perform third generation rapid assay by FREND™ COVID-19 Ag, NanoEntek, Inc. and PCR assay by RT-LAMP technology, SARS-CoV-2 POC, Enbiotech srl.

Results. The workflow includes:

1- Patient identification by an healthcare assistant
2- Anamnesis and nasal swab by a doctor and a nurse
3- Performing a third generation rapid assay by a laboratory technician and a biologist
4- Positive patients are immediately subjected to a nasopharyngeal swab by a nurse
5- Performing PCR assay by a laboratory technician and a biologist
6- Patient health care by a doctor

Discussion and conclusions. The absolute novelty of this center is represented by the on-site presence of laboratory staff. This allows to have immediate and safe confirmation results thanks to the competence of those who perform them. The presence of different professional figures guarantees the asymptomatic patient the optimal management of SARS-CoV-2 infection.

54 - Molecular characterization of the emerging Streptococcus pneumoniae serotype 8 from invasive isolates

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Introduction: Serotype 8 *Streptococcus pneumoniae* (S8*Sp*) is a frequent cause of invasive pneumococcal diseases (IPDs) in adults and elderly. The ST53 major clone, has been detected worldwide and emerging cause of outbreaks in Europe. Aim of this study was to investigate the molecular characteristics of S8*Sp* causing invasive infections at "A. Manzoni" hospital, Northern Italy. **Materials and Methods:** During the three-year period 2017-2019, n=21 S8*Sp* have been collected from blood samples at Lecco "A. Manzoni" acute care hospital. Bacterial identifications and antimicrobial susceptibility tests were obtained by MALDI-TOF mass spectrometry and Vitek2 System (bioMérieux), respectively. Serotyping was provided by the Pneumococcal Regional Reference Laboratory (Milan, Italy). The virulence genes content was investigated by targeted PCR, for the genes: *lytB*, *lytC*, *cbpC*, *cbpD*, *cbpE*, *cbpF*, *cbpG*, *cbpI*, *cbpJ*, *pcpA*, and *pspA*. Molecular characterization included the MultiLocus Sequence Typing (https://pubmlst.org/organisms/streptococcus-pneumoniae) and the RAPD analysis (kit Ready to go, Merck). **Results:** Ten/21 isolates were collected from inpatients at Medicine, 5/21 Emergency, 3/21 Oncology and the three remaining at other wards (Dialysis, Nephrology and ICU). The 21 isolates were susceptible to all the antibiotics tested. Ten/21 strains (47.6%) were positive for both *lytB* and *lytC* genes, 5/21 strains (23.8%) were negative for both genes, 5/21 strains (23.8%) were positive only for *lytC*; one strain (4.7 %) was positive for *lytB* only. All the strains harbored *cbpC* and *pcpA* genes; 17/21 isolates (81%) were *cbpI* positive. The *cbpD* and *cbpE* genes were instead detected in 15/21 strains (71.4%). Two/21 strains (9.5%) were *cbpF* and *cbpG* positive, while *cbpJ* was found in one strain only (4.7%). The *pspA* gene was not detected. Seven STs were overall identified: ST53 was the most represented (15/21; 71%), and spread in Medicine (6/15; 40%), Emergency (5/21; 33%), Oncology (3/15; 20%) and ICU (1/15; 7%) wards. Five/21 strains belonged to the already known ST193, ST404, ST433, ST944, ST15126, while the remaining isolate showed a new ST profile. The ST53 was successful discriminated by the two primers 2 and 6 of the RAPD kit, thus providing a faster and less costly/labor-intensive alternative for epidemiological surveillance of the most virulent 53ST. **Discussion and Conclusions:** ST53 is associated with a greater ability to cause outbreaks and IPDs in the adult population. Our results confirmed the broader virulence genes pattern and pathogenic potential of the ST53 S8*Sp* strains. The "two-primers" RAPD approach used can represent an intriguing method bridging the "typing gap" between serotype and sequencing.

119 - Rapid identification of nontuberculous mycobacteria directly from positive primary MGIT coltures by MALDI-TOF MS

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Introduction: Over the last years, nontuberculous mycobacteria (NTM) have emerged as important human pathogens. Accurate and rapid mycobacterial species identification is needed for successful diagnosis, treatment, and management of infections caused by NTM. Matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) was demonstrated to effectively identify mycobacteria isolates subcultured from solid or liquid media rather than new positive cultures. The aim of the present study is to develop a rapid method for direct identification of NTM from primary MGIT cultures by MALDI-TOF MS.

Materials and Methods: A total of 20 positive MGIT broths, collected from February to July 2021, were examined by the Bruker Biotyper system with Mycobacteria Library v 2.0 (Bruker Daltonics). Extraction was performed within 24-72 h after automated growth detection by MGIT. Protein extraction was carried out by the manufacturer's MycoEx protocol. Results were compared with those obtained by the Line probe assay GenoType Mycobacterium CM/AS/NTM-DR (Hain LifeScience).

Results: Our results showed concordant identification for all the mycobacteria isolated. In particular, the molecular test identified the mycobacteria as *M. avium* (n. 5), *M.intracellulare* (n. 3), *M. chimaera* (n. 3), *M. gordonae* (n. 2), *M. fortuitum* (n. 2), *M. tuberculosis* complex (n. 2), *M. chelonae* (n. 1), *M. lentiflavum* (n. 1) and *M. celatum* (n. 1). All identifications based on MALDI-TOF MS had scores >1.7 and were concordant with the molecular identifications. MALDI-TOF MS cannot differentiate between *M. intracellulare* and *M. chimaera*, two closely related potentially pathogenic species of NTM that are members of the *M. avium* complex.

Discussion and conclusions: Although a small number of strains and a limited diversity of mycobacterial species were analysed, our results indicate that MALDI-TOF MS could represent a useful routine diagnostic tool for identification of mycobacterial species directly from primary liquid culture.

91 - Discriminatory Weight of SNPs in Spike SARS-CoV-2 Variants. A technically rapid, unambiguous and bioinformatically validated laboratory approach

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Introduction In the continuous struggle among organisms, variability is the key to success. This is even more true for viruses, as they have to change their genome to respond to the host's immune system and become resistant to drugs. Nowadays, new important SARS-CoV-2 lineages descending from the first Wuhan strain and with increased transmissibility have been discovered. In this scenario, laboratories should try to identify the presence of already known - and even unknown – mutations, at least in the viral Spike (S) gene. This information alone is not discriminant, and samples sequencing is the only way to ascertain the presence of mutations and deletions in the gene. In this work, we describe a rapid, adaptable, and affordable workflow to detect all mutations related to the SARS-CoV-2 lineages described by the World Health Organization (WHO), based on two PCRs and two Sanger sequencing reactions. In fact, all variants harbor both "unique" SNPs and shared mutations. To avoid errors, it is therefore necessary to sequence SARS-CoV-2 portions containing a unique and discriminating combination of SNPs that should not be randomly chosen, but rather selected based on cluster and identification.

Materials and Methods Viral RNAs from 80 samples from different structures in Eastern Sicily were extracted, retro-transcribed and subjected to PCR amplification and subsequent Sanger sequencing. Instead of sequencing the entire S gene, only a few portions called Fragment Spike (FS) were selected. To characterize mutations related to the different SARS-CoV-2 lineages, we arbitrarily divided the S gene into 8 portions and designed a pair of primers on each portion and a pair of primers for the N gene useful to identify the Nextstrain cluster 20A.EU2 clone.

Results For the rapid and unambiguous identification of the variants of SARS-CoV-2, it was appreciated how the gene portion between FS-4 and FS-5 contains the defining SNPs that allow the univocal identification of the reported variants. On each portion of the S gene, three parameters (Mutational Percentage Explained $\%_{MUT}$, Degree of Uniqueness U, Mutational Density d_{MUT}) were calculated that express not only the mutational density of the portion itself, but also the quality and weight of the mutations.

Discussion and Conclusions The three parameters allowed us to state that a Hot Spot Mutation Fragment exists on the S gene that allows the identification of defining SNPs through a single PCR reaction with appropriate primers. The combination of SNPs - and the consequent formation of genetic clusters - is crucial in SARS-CoV-2 variant identification. The chosen portion of the SARS-CoV-2 S gene contains a set of SNPs which are useful to discriminate many variants uniquely, rapidly and with few reactions.

66 - Reverse transcriptase loop-mediated isothermal amplification (RT-qLAMP) as a user-friendly system to detect SARS-CoV-2 infection: a multicentric study

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Introduction: Quantitative reverse transcription PCR (RT-qPCR) is considered the standard for viral detection, but more rapid and affordable diagnostic tests are required to identify COVID-19 infection. Real-Time loop-mediated isothermal amplification coupled with reverse transcription (RT-qLAMP) is a more rapid, cheap, and easy method for diagnostic tests that has some benefits compared to RT-qPCR, including limited dependency on expensive instruments, fast processing time, and user-friendly interpretation of results. Herein, we report on the accuracy of an Italian RT-qLAMP diagnostic kit for SARS-CoV-2 diagnosis developed by Enbiotech SRL (Palermo, Italy) through the analysis of the data collected in the first Sicilian multicentric study comparing RT-qLAMP and RT-qPCR results.

Material and Methods: 551 nasopharyngeal specimens collected from patients admitted to Sicilian Hospitals were processed by RT-qLAMP and RT-qPCR. Each tested sample was recorded in a datasheet providing the following information: ID, date and aim of the test, RT-qLAMP results reporting the amplification curve timepoint, RT-qPCR system name and results reporting the threshold cycle (Ct) values for each of the three amplified genes, and overall result (positive or negative) for both RT-qLAMP and RT-qPCR. Statistical analyses were performed calculating sensitivity, specificity, positive and negative predictive values comparing SARS-CoV-2 POC kit for RT-qLAMP with RT-qPCR results.

Results: According to our results, 39.9% and 41.1% of samples tested positive for SARS-CoV-2 by RT-qLAMP and RT-qPCR analysis, respectively. This small discrepancy was statistically investigated by calculating the sensitivity, specificity, positive and negative predictive value of the SARS-CoV-2 kit for RT-qLAMP by comparison with the data obtained with RT-qPCR. These analyses revealed a sensitivity and specificity of ≥95% and strong positive and negative predictive values (98% and 97% respectively) indicating the reliability of the method.

Discussion and Conclusions: These results suggest that RT-qLAMP could be a valid diagnostic alternative to detect SARS-CoV-2 positive patients where and when there is a need for rapid yet reliable tests that can be performed without the use of expensive instruments and reduce any potential user-dependent biases. The "one-step" approach requires less than 90 minutes from sampling to obtain the results and it avoids the need for other kits and instruments to perform RNA extraction and reverse transcription. The data presented in this work highlight that RT-qLAMP has a good diagnostic power and it may be useful for the implementation of a Point of Care in settings requiring rapidity and reliability without using expensive instruments and high skilled personnel.

04 Resistenza agli antimicrobici, sorveglianza e test di sensibilità

120 - Activity of ceftolozane/tazobactam and comparator antibiotics against a nationwide collection of extended-spectrum cephalosporin-resistant Enterobacterales

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Introduction
Ceftolozane/tazobactam (CTZ) is an extended-spectrum cephalosporin/beta-lactamase inhibitor combination able to combat infections by multi-drug resistant Gram-negatives. CTZ shows a poor activity against class A and class B carbapenemases, while retains a good efficacy against extended-spectrum β-lactamase (ESBL) producing *Enterobacterales*. In this study we evaluated the activity of CTZ and comparators against a nationwide collection of clinical isolates of extended-spectrum cephalosporin-resistant (ESCR) *Enterobacterales*.

Materials and methods
A collection of clinical isolates of *Enterobacterales* with a MIC >1 mg/L to at least one ESC collected during a late 2013 national cross-sectional survey (534 non replicate isolates, including 386 *Escherichia coli*, 82 *Klebsiella pneumoniae*, and 66 *Proteus mirabilis)* was included in the study. All isolates have already been characterized phenotypically and partially genotypically for CTX-M-type ESBLs and acquired class C beta-lactamases.

Antimicrobial susceptibility testing was carried with broth microdilution method (BMD) using lyophilized plates (Trek Diagnostic Systems, US) and CTZ also with MIC Test Strip (MTS, Liofilchem, Italy), starting from the same inoculum. BMD and MTS results were compared following ISO 20776-2:2007 guidelines. Results were interpreted following EUCAST clinical breakpoints (v.11.0, 2021).

Results
CTZ resulted susceptible for 85.3% of tested isolates (93.3% of *E. coli*, 68.3% of *K. pneumoniae*, 60.6% of *P. mirabilis*) compared to 99.6% of meropenem, 98.3% of colistin, 93.4% of amikacin, 69.9% of piperacillin-tazobactam, 58.7% of gentamicin, 36.9% of trimethoprim/sulfamethoxazole, 21.7% of amoxicillin-tazobactam and 12.4% of ciprofloxacin. CTZ showed a higher activity against ESBL-producing *E. coli* compared to CMY-producing *E. coli* (94.0% vs 71.4%, respectively) a good activity against ESBL-producing *P. mirabilis* (87.8%), while resulted less effective against ESBL-producing *K. pneumoniae* (64.9%) and CMY-producing *P. mirabilis* (12.5%).

MTS results compared with BMD for CTZ showed good category agreement and essential agreement (499/534, 93.4% and 486/513, 94.7%, respectively), an acceptable number of major errors (4/456, 0.9%), but a significative underestimation tendency with a consistent number of very major errors (31/78, 39.7%).

Discussion and conclusion

CTZ showed a potent activity against ESCR *Enterobacterales*, but with a marketed higher efficacy against *E. coli*, compared to *K. pneumoniae* and *P. mirabilis.* MTS showed a poor reliability to detect resistant isolates of *Enterobacterales* with an important underestimation tendency. The evaluation of the activity of CTZ on a more recent collection of *Enterobacterales* would be of interest.

163 – Drug-repurposing for SARS-CoV-2 infection: antiviral potential of dihydroartemisinin-bile acids conjugates

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Introduction

The global COVID-19 pandemic highlighted the need of rapid methods and technologies for containing the spread of SARS-CoV-2. While vaccines will prevent disease occurrence, infected individuals still need treatment options, and drug repurposing can provide a rapid and potential cure toward emerging infectious diseases.

The antimalarial agents, artemisinins, as well as their synthetic versions have shown activity against several DNA and RNA viruses. Among others, dihydroartemisinin (DHA) can be easily conjugated to lipophilic moieties such as bile acids, that have been found to bind to the spike protein of the SARS-CoV-2. In our work, we focused on testing antiviral activity of different conjugates of DHA and bile acids against SARS-CoV-2.

Materials and Methods

Three different DHA-conjugates were synthetized and selected for testing antiviral activity.

Cytotoxicity assay (MTT) was performed to identify the non-toxic dose in human cell lines. For the time-drug-addiction, cells were treated with conjugates at different steps of infection (viral absorption, replication). Antiviral activity of conjugates was assayed on SARS-CoV-2 infected VeroE6 cells and evaluated by rt-qPCR. IC50 values were identified by fitting the inhibition curves to the data using nonlinear regression analysis.

Results

The conjugates (from 0.1 to 100 µM, serial dilutions) were tested on human cells (MRC5) for the evaluation of cytotoxicity by MTT and found to be not toxic up to 10 µM. Then, 1 and 10 µM of DHA conjugates were selected to treat VeroE6 cells during the infection with SARS-CoV-2 (100 genome equivalent per cell). The results showed that conjugate composed by ursodeoxycholic acid's methyl ester (UDCOMe) linked to DHA via triazole moiety (DHA-t-UDCOMe) was able to decrease the viral load of 1.87 logs after 24 hours post infection (hpi) and 2.735 logs after 48 hpi, with respect to the untreated infected cells. Time-drug-addiction assay demonstrate that DHA-t-UDCOMe was able to reduce viral load at both viral absorption and replication steps.

Discussion and Conclusions

These preliminary results suggested that DHA-t-UDCOMe has a potential antiviral activity against SARS-CoV-2. Previous works reported the broad-spectrum antiviral potential of artemisinins, while bile acid derivatives target spike protein inhibiting the interaction with host receptor. The proposed mechanism of action for DHA-t-UDCOMe is that the combination of dihydroartemisinin with bile acids in a rigid amphipathic structure could affect viral entry in a dual mode: by disrupting envelope organization and by affecting the binding to cellular receptors. Further studies are needed to determine the mechanisms of action of DHA-t-UDCOMe and to characterize the potential antiviral action in human tissues.

9 - Global distribution of 16S rRNA methyltransferases in NDM-producing Klebsiella pneumoniae: Which kind of future for aminoglycosides against NDM?

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Introduction The diffusion of carbapenem resistance is a global health threat and the spread of the NDM carbapenemase, initially described in patients receiving healthcare in India, is of particular concern. Aminoglycosides are an important second line therapeutic choice for infections caused by carbapenem resistant Enterobacterales, and great hopes lie in the development of new generation aminoglycosides (Neoglycosides), since these presumably will be not affected by the most diffused resistance mechanisms to aminoglycosides (the aminoglycosides modifying enzymes). Yet, even these molecules, could be rendered ineffective by 16S rRNA methyltransferases (16RMTases), which act by modifying the active site on which aminoglycoside operate. Not knowing the epidemiology of 16RMTases, both from a local and a global point of view, poses a great threat to the use of all aminoglycosides. Therefore, starting from the analysis of an outbreak of 9 NDM and rmtC co-producing *K. pneumoniae* occurred at our hospital, a study was undertaken to characterize the 16RMTases diffusion and its association to the *bla*$_{NDM}$ gene.
Materials and Methods Out of the 9 NDM-producing strains, 4 were subjected to WGS using both short (Illumina) and long (Nanopore) reads and analyzed with several *in silico* tools (e.g. ResFinder, PlasmidFinder, Kleborate). Furthermore, a search of NDM- and of 16RMTases-producing *K. pneumoniae* was performed on the GenBank global database.
Results Our strains belonged to the high risk ST15 clone, did not carry any virulence gene and presented the *aac(6')-Ib3*, *sul1*, Δ*qacE*, *rmtC*, *bla*$_{NDM}$, *ble*$_{MBL}$ and *bla*$_{CMY-6}$ resistance genes scattered alongside an incompatibility type C (IncC) plasmid. *bla*$_{CMY-6}$ can be found between an IS*Ecp1* and the *blc-sugE* genes, while *aac(6')-Ib3*, *sul1*, Δ*qacE*, *rmtC*, *bla*$_{NDM}$, *ble*$_{MBL}$ are located in the ARI-A of the IncC plasmid. Specifically, *rmtC* and *bla*$_{NDM}$ are located 1246 bp one from the other in a tail-tail configuration, separated by an IS*Kpn14* which disrupted the IS*Aba125* that is typically associated with *bla*$_{NDM}$. Analyzing the diffusion of all 16RMTases in 91 NDM-producing *K. pneumoniae* present in the GenBank database, we noted how that 48,3% of these strains (44/91) co-harboured at least one 16RMTase.
Discussion and Conclusions The association between *bla*$_{NDM}$ and 16RMTases is more and more tangible, and this puts in great risk the use of aminoglycosides against the most diffused metallo-beta-lactamase. The spread of 16RMTases and *bla*$_{NDM}$ is mediated by several different *K. pneumoniae* STs, equivalently to the spread of this metallo-beta-lactamase alone, suggesting the possibility that the use of aminoglycosides should be limited to the cases in which there is a proven susceptibility, but cannot be used as a support empiric treatment.

77 - Two years environmental surveillance of Legionella spp. in raylway buildings

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Introduction: *Legionella* spp. is the causative agent of Legionellosis or Legionnaires' disease. This bacterial species prefers natural and artificial basins, buildings water system, temperatures between 25° and 42° C° and it is transmitted to human through the inhalation of contaminated aerosols. Legionnaires' disease is a lung infection similar to pneumonia, with symptoms that include cough, fever, asthenia, after an incubation period of 2-10 days. All places involved to exposure water spray have to be considered at risk; water quality monitoring and control are essential to prevent disease in public buildings. Our surveillance demonstrates the constant presence of *Legionella* strains in the water system under control, indeed it is necessary to carry out environmental surveillance to safeguard the health of workers. In the last 2 years, we processed in the reference laboratory 81 samples of hot and cold water from railway buildings, coming from 8 different sicilian areas.
Materials and methods: The water samples arrived at the laboratory in sterile 1 liter bottles, represented by hot and cold water from washbasins, showers, dressing room of the raylway sicilian buildings. Samples have been analyzed by referring to the protocol indicated in the Italian guidelines, the filtering of the water took place through membranes of 0.2 µm. *Legionellae* grow on several types of complex artificial media, however, the most successful medium and procedure include buffered charcoal yeast extract (BCYE) agar. Most isolates demonstrate growth in 3 to 5 days.
Results: The analyzes showed the presence of the microorganism in all areas, of 81 collected water samples, 44 (54,3%) were positive. In particular, more than half of the contaminated samples, 52,2% were positive for more than one serogroup or species (*Legionella anisa*). Regarding positive water samples, most of them were contaminated by *Legionella pneumophila* sgr 1. Almost all the samples had a bacterial load in the range between 100-1000 CFU/L.
Discussion and conclusions: Our results demonstrate the presence of the microrganism in all the areas analized, quantifyng through the water system the microbial load of the bacterium. Moreover, we confirm the presence at the same time of other *Legionella* species associated to *L.pneumophila*. Obviously, should be appropriate to avoid possible exposure of workers in the contaminated raylway buildings, using new quick and easy tests or procedure, to detect *L.pneumophila*, the most dangerous causitive agent of the *Legionella* genus, but also other less common species.

86 - Clinical carbapenem-resistant Acinetobacter spp. in pet animals: an exploratory study

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1. Introduction. Many studies have been performed on carbapenem-resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex and only a few studies have been addressed on carbapenem resistance in not-ACB complex species. *A. baumannii* infections are an important concern in human and veterinary medicine, considering its ability to cause invasive infections and acquire multidrug resistance. Recently, colistin and carbapenem-resistant *A. baumannii* have increased worldwide with a reduction of the antimicrobial efficacy, especially in case of coinfection with the pandemic SAR-CoV-2. To assess the frequency and the antimicrobial susceptibility of both ACB and not-ACB complex members, an epidemiological study on pet animals was carried out.
2. Materials and Methods. From 2018 to 2020, different clinical speciments from dogs, cats and horses were cultured and *Acinetobacter* spp. identified by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Susceptibility to a panel of 14 human and veterinary antimicrobials, belonging to 9 different categories, was assessed by Kirby-Bauer and E-test methods.
3. Results. From respiratory, enteric, genital, urinary, skin, joint-bone, and sistemic infections of clinically hospitalized and in-visit pet animals, 628 specimens were cultured and *Acinetobacter* strains (n=16, 2.5%) were identified. The same percentage (50%) of ACB complex members (*A. baumannii*, *A. dijkshoorniae*, *A. pittii*) and not-ACB complex members (*A. lwoffii*, *A. courvalinii*, *A. johnsonii* and *A. bereziniae*) was observed. The nosocomial origin was significantly represented (P=0.045) in not-ACB complex strains. All *Acinetobacter* spp. were sensitive to aminoglycosides and polymyxins. The same carbapenem-resistance (37.5%) was observed for both groups, involving *A. baumannii* and *A. dijkshoorniae* for the first group, *A. courvalinii* and *A. bereziniae* for the second one. ACB complex strains showed significant higher resistances for tetracyclines (P=0.021) and monobactams (P=0.007).
4. Discussion and Conclusions. *Acinetobacter* isolates belonging to the ACB and not-ACB complex are of particular concern in pet animals since these bacterial species are also associated with the nosocomial and in-visiting clinical settings. The two isolated multidrug-resistant *A. dijkshoorniae* strains represent the first identification in clinically infected dogs in Italy. Phenotypic antimicrobial resistance, in addition to the carbapenem resistance observed for ACB and not-ACB strains, can be considered a public health concern. In particular, not-ACB complex species, showing relevant resistance profiles, strengthen their role as zoonotic pathogens.

58 - Synergistic activity of a synthetic 1,2,4-oxadiazole-containing derivative and oxacillin against methicillin-resistant Staphylococcus aureus

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Introduction: *Staphylococcus aureus* represents an important opportunistic pathogen able to cause many infections in humans and animals as skin or lung infections, endocarditis, toxic shock syndrome, and sepsis. The inappropriate use of antibiotics has favoured the diffusion of resistant-bacteria, as for beta-lactams and the selection of methicillin-resistant *S. aureus* (MRSA), that has nullified the efforts done in the discovery of antimicrobial agents. Thus, the discovery of new compounds potentially able to control diseases by resistant bacteria and restore the activity of existing antibiotics represents an urgent goal. Oxadiazole nucleus is a relevant scaffold in organic and medicinal chemistry for its elevated versatility to afford molecular diversity, and for therapeutic potential. Previously, a wide library of oxadiazole-containing derivatives was discovered as potent antibacterial agents against multidrug-resistent MRSA strains. Our study is aimed to further expand the structural diversity around the selected scaffold in order to define the antibacterial activity.
Materials and Methods: A library of 1,2,4-oxadiazoles was synthetized based on amidoxime and carboxylic acid heterocyclization. The synthetized compounds were characterized by a common 1H-indol-4-yl substitution at C5 and a variable substituted aromatic ring at C3 on the heterocycle ring. The compounds were tested for their antimicrobial activity against *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300 (MRSA) by the broth microdilution assay. The synergism between A16 and oxacillin against MRSA was determined using the checkerboard technique.
Results: The compounds were tested for their antibacterial activity and possible synergistic effects in combination with oxacillin. The strongest antimicrobial activity was obtained with the compounds E16 (4 mM) and A16 (2 mM). The time kill assay confirmed the bactericidal activity of A16. Then, we investigated the ability of A16 to improve the activity of oxacillin against the MRSA (MIC for oxacillin 10mg/ml). The highest synergistic interaction was obtained with the combination values of 0.78 μM A16 and 0.06 μ*g*/ml oxacillin. The FIC index value of 0.396, confirmed the synergistic effect of A16 and oxacillin. Finally, the compound resulted not cytotoxic at the MIC value.
Discussion and conclusion. The results reported are of interest since we demonstrated the ability of A16 to arrest the growth of the MRSA and to restore the activity of oxacillin lowering the MIC value, well below the breakpoint value (≤ 2 μg/ml) recommended by CLSI. Inconclusion, A16 may be promising in the treatment of resistant infection by MRSA. Studies are in progress to analyse its ability to modulate the expression of *mec* operon genes.

47 - GENOMICS OF A CLINICAL DAPTOMYCIN RESISTANT VISA CA-MRSA

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BACKGROUND
Daptomycin-Resistant (DAP-R) Vancomycin-Intermediate (VISA) Community-Acquired (CA-) MRSA represents a serious challenge for its infection therapeutical treatment. CA-MRSA, having the small IV and V SCC*mec* locus, is commonly hypervirulent – harboring also the PVL toxin gene – and antibiotic-resistant only versus β-lactams and macrolides.
METHODS
Genomics of a DAP-R and VISA CA-MRSA was investigated comparing the phenotypes and genotypes of the DAP-R VISA CA-MRSA versus its Daptomycin-Susceptible (DAP-S) and Vancomycin-Susceptible (VSSA) CA-MRSA parent using Whole-Genome Sequencing (Illumina MiSeq Platform) and Bioinformatics. Genomic epidemiology, SNPome, virulome, resistome and antibiotype were analyzed. Single-Nucleotide Polymorphism (SNP) calling were performed mapping on the genomically-related *S. aureus* MW2 (CA-MRSA) Reference Genome.
RESULTS
Our DAP-R VISA CA-MRSA was ST1-spatypet127-*agr*III-SCC*mec*IVa, with *rep*5a (pN315), *rep*7a (*rep*C-Cassette), *rep*7c (MSSA476), *rep*10 (pDLK1), *rep*16 (pSAS) plasmid genes and a huge content of phage-encoded toxin genes including *luk*S/F, *sea*, *sec*, *seg*, *seh*, *sei*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *tst* as well as resistant to cefoxitin (FOX), DAP and VAN intermediate resistance. Resistomics evidenced the aminoglycoside-R *ant*(6)-Ia and *aph*(3')-III, β-lactam-R *blaZ*, β-lactam-R *mec*A, macrolide-R *erm*C and tetracycline-R *tet*K consistent with the strain antibiotype. Furthermore, comparative SNPomics showed 21 non-synonymous SNPs in DAP-R VISA CA-MRSA versus its VSSA/DAP-S CA-MRSA parent, including *agr*A, *tpi*A, and *spa*, with a disruptive impact on the related protein. "Already-known" nsSNPs -associated to rifampicin RIF/VAN, DAP, and Fluoroquinolone resistance- were found in *mpr*F (T345A), *rpo*B (H481Y), in *gyrA* (S84L), and in *grl*A (S80F), respectively.
CONCLUSION
Genomics highlighted the huge hypervirulence and the awesome extended antimicrobial resistance, especially towards the last-resort antimicrobials, of our clinical DAP-R VISA CA-MRSA superbug.

78 - Effects of Penicillin-Binding Protein 4 (PBP4) alterations on ceftobiprole activity against Enterococcus faecalis clinical isolates

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Introduction: Penicillin-resistant ampicillin-susceptible *Enterococcus faecalis* strains (PRAS) have been associated with overexpression or aminoacidic substitutions in the low-affinity penicillin-binding-protein 4 (PBP4), a phenomenon of some concern as synergistic combination with cell wall-active agents is frequently used in the treatment of enterococcal infections. Ceftobiprole (BPR), a new-generation cephalosporin, is a therapeutic option against *E.faecalis.* Our aims were to investigate the *in vitro* antibacterial and bactericidal activity of ceftobiprole and its synergism with beta-lactams against *E.faecalis* clinical isolates belonging to different antibiotic-resistance classes; to analyze the possible occurrence of *pbp4* gene mutations and consider their role in BPR antibacterial and *cidal* activity, in MDR phenotypes of β-lactam resistance.
Materials and Methods: Twenty-two clinical *E. faecalis* strains from bloodstream infections (BSIs) were analyzed for their antibiotic susceptibilities by the broth microdilution (BMD) method. BPR bactericidal and synergistic activities by time-kill curve assays and *pbp4* gene sequence analysis were assessed against selected MDR-*Efs* representatives of different antibiotic-resistance and β-lactam resistance profiles.
Results: Time-kill experiments demonstrated a potent BPR bactericidal activity (3-4 $\log_{10}$ reduction) despite their MDR-*Efs* classes and PBP4 alterations, also after 8h. A synergistic effect in combination with all beta-lactams tested was shown in 3 of the most resistant strains. *pbp*4 sequence analysis revealed the deletion of an adenine in the promoter region of four BPR-NS strains with high-level penicillin resistance, responsible for the reduced susceptibility to BPR, and resistance to other beta-lactams. Translated sequence analysis identified diverse missense mutations in the PBP4 non-penicillin-binding domain. In only one BPR-NS strain, the combination of the adenine deletion in the promotor region and a T418A substitution near the catalytic site highly affected BPR MIC value (BPR 16mg/L).
Discussion and Conclusions: Ceftobiprole showed a promising *in vitro* antibacterial and bactericidal activity against *E. faecalis* strains, due to its high affinity for PBP4. Our *in vitro* dynamic analysis by time-kill curves showed that BPR exerts a bactericidal activity against *E.faecalis* despite their MDR phenotypes and a synergistic effect in combination with β-lactams against VRE, PRAS and BPR-NS isolates. A comprehensive evaluation of the role of *pbp*4 molecular alterations against MDR-*E. faecalis* strains belonging to different antibiotic-resistance and β-lactam resistance profiles showed that they might influence the β-lactam-susceptibility values without affecting BPR *cidal* activity.

141 - Sesquiterpene lactones from Cotula cinerea with antibiotic activity against clinical isolates of Enterococcus faecalis

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INTRODUCTION - A remarkable interest has occurred in the last twenty years for the rediscovery of natural substances as a potential reservoir of innovative therapeutic solutions for human health, with the prospect of integrating and sometimes replacing conventional drugs. In particular, there is a growing interest in the study of natural substances extracted from plants to counter the galloping phenomenon of antibiotic resistance and as a possible alternative in the fight against microbial biofilm, a structure consisting of an extracellular matrix that incorporates the bacterial community that formed it, acting as a barrier for antimicrobial agents and conventional antibiotics.
Cotula cinerea belonging to the tribe Anthemideae is a plant widespread in Southern hemisphere. It is frequently used in folk medicine in North Africa countries for the several medical properties showed by its extracts and essential oils. The dichloromethane extract obtained from its aerial parts demonstrated antibiotic activity against *Enterococcus faecalis*. Thus, it was fractionated and the pure metabolites obtained were tested for their antibacterial and antibiofilm activity against clinical strains of *E. faecalis*.
MATERIALS AND METHODS - The CH_2Cl_2 extract was fractionated by bioguided purification procedures affording five main sesquiterpene lactones. They were identified by spectroscopic methods. The absolute configuration was assigned by applying the advanced Mosher's method to haaegenolide and by X-ray diffraction analysis to 1,10 –epoxyhaagenolide. The specific antibiotic and anti-biofilm activity were tested towards *E. faecalis* clinic isolates with plate microdilution method and crystal violet assay.
RESULTS: NMR and ESIMS data identified five metabolites as the guaiantrienolides 6-acetoxy-1beta-, 6-acetoxy-1alpha-, and 6-acetoxy-10-beta-hydroguaiantrienolide (1-3) and the germacrenolides haagenolide and 1,10–epoxyhaagenolide (4 and 5). The results showed that compounds 3-5 have antibacterial activity against all the strains of *E. faecalis* while compound 2 exhibited activity only towards some strains. Compound 1 did not show this activity but had only antibiofilm properties.
DISCUSSION AND CONCLUSIONS - This is the first report on the antimicrobial and antibiofilm activities of three guaiantrienolides and two germacrenolides isolated from *C. cinerea* against clinical isolates of *E. faecalis*, a common drug-resistant opportunistic pathogen responsible of important biofilm-related infections. Our results are preliminary data, but we can hypothesize that these natural substances could be a potential alternative for new antibiofilm formulations in strategies for the prevention of persistent infection by *E. faecalis*.

60 - Evaluation of the antifungal activity of selected essential oils on Aspergillus spp. and Candida spp.

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Introduction. Microorganisms have a remarkable ability to develop resistance to antimicrobial agents, threatening the effectiveness of drugs and becoming a serious public health problem. This is more evident for fungal infections that can therefore sometimes be difficult to treat. Currently, new trend in drug discovery to overcome this problem is represented by natural products from plants, or their extracts. Particularly, there is a great interest in essential oils (EOs) recognized for their antimicrobial role towards bacteria, fungi and viruses. In this study, we evaluated the antifungal activity of several commercial EOs (Flora, Italy) in comparison with fluconazole (FLC), itraconazole (ITZ), voriconazole (VRZ) and amphotericin B (AMB) against *Candida krusei, C. valida*, *Aspergillus fumigatus* and *A. niger*, clinical isolates that continue to increase as the population of immunocompromised patients increases.
Materials and Methods. Commercial EOs of clove (*Eugenia caryophyllata*), geranium (*Pelargonium asperum*), oregano (*Origanum vulgare*), lavender (*Lavandula vera*) and thyme red (*Thymus vulgaris*) have been screened for antifungal activity against 6 *A. fumigatus*, 5 *A. niger*, 6 *C. krusei* and 6 *C. valida* strains. Antifungal activity was determined by broth microdilution method, and, for *A. fumigatus* and *A. niger* strains, also by aromatogram. FLC, VRC, ITZ and AMB were used as positive controls. Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) of EOs and drugs were evaluated according to the CLSI, with some modifications for EOs. The final EOs concentrations ranged from 1-0.078% (v/v).
Results. All aspergilli tested were resistant to ITZ and/or AMB (breakpoint R>2µg/ml). All *C. krusei* strains were resistant to VRC, while on the contrary all *C. valida* strains were resistant to FLC. Geranium and clove EOs showed the highest antifungal activity against all tested strains with geranium's MIC ranges of 0.06-0.25% (v/v) and clove's MIC ranges of 0.125-0.25% (v/v). As regard *C. valida* and *C. krusei*, uncommon but increasing pathogen yeasts, thyme red showed the highest activity *in vitro*, with MIC ranges of 0.06-0.12% (v/v). MFC was 1-2 times higher than MIC, indicating a fungistatic action of the tested OEs especially against *A. fumigatus* strains. Aromatogram confirmed the antifungal activity of these two OEs, showing significant growth inhibition.
Discussion and Conclusions. The data obtained indicate a good antifungal activity of some OEs against candida and aspergillus strains resistant to conventional drugs. The results of this work support the research for new alternatives or complementary therapies based on EOs.

27 - In vitro evaluation of virucidal activity of nanostructured antimicrobial coatings for high-touch surfaces

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The SARS-CoV-2 virus, responsible for ongoing pandemic, is transmitted through respiratory droplets as well as through contaminated surfaces. Most of the available sanitation procedures are based on UVC light or chemicals, but these methods may be associated with toxicity and/or materials damage. Therefore, new effective and safe strategies to inactivate viruses on environmental surfaces are needed. The aim of our study is to assess the *in vitro* virucidal activity of two newly synthesized nanostructured antimicrobial materials (NAMs) developed to coat high-touch surfaces. We here present the data obtained with Human Coronavirus-OC43 (HCoV-OC43), Herpes Simplex Virus-1 (HSV-1) and Adenovirus-5 (AdV-5), chosen for their different environmental resistance. The NAMs were obtained by dissolving chitosan powder into an aqueous solution of acetic acid (NAM1) or polycaprolactone granules into chloroform (NAM2) and subsequently by adding in both polymer solutions chlorohexidine-based nanoparticles as active agent. These dispersions were used to coat polypropylene substrates which were contaminated with the different viruses. At different time points after contamination, the residual virus was recovered and quantified by the end-point titration method. Both NAMs showed a virucidal activity > 2 Log against HCoV-OC43 and HSV-1 starting after 6 h, with a further increase after 24 h. No significant activity was observed against AdV-5

Our preliminary results suggest that nanostructured coatings may be proposed as an effective alternative to traditional disinfection of environmental surfaces potentially contaminated by viruses.

111 - Antiviral properties of a probiotic-based detergent system able to prevent antimicrobial resistance selection: implications for COVID-19 prevention

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Introduction: The ongoing COVID-19 pandemic has highlighted the need for effective decontamination sanitation procedures, but these should consider the even larger threat of spread of antimicrobial resistance (AMR), already killing hundreds of thousands of people around the world and often complicating the care of COVID-19 patients. Indeed, the sanitation procedures based on high concentrations of chemical disinfectants show limitations, as they have a temporary effect, high environmental impact, and might further spur AMR.
Based on previous results obtained by our group showing that an eco-sustainable sanitation system involving remodulation of hospital microbiome by selected probiotic *Bacillus* could stably reduce resistant pathogens (-80%) and related infections (-52%), the aim of this study was to assess the antiviral properties of such system.
Materials and Methods: *In vitro* tests were performed to assess the antiviral activity of the probiotic-based sanitation (PBS) against enveloped viruses, including human coronavirus 229E, vaccinia virus, herpesvirus type 1, flu viruses (human and animal strains), and SARS-CoV-2. PBS activity was analyzed both in solution and on hard non-porous surfaces, at different concentrations and times of contact, evaluating the effectiveness both in removing and preventing virus contamination.
Results: PBS significantly decreased the virus contamination in solution and on surfaces, with times of application comparable to those required for antiviral action certification of products (UNI EN 14476, UNI EN 16777:2019). Notably, the system could induce a >4Log decrease of virus load in maximum 2 hours, depending by the virus type. Full action was observed against SARS-CoV-2 and even against the most resistant enveloped viruses (vaccinia virus). Notably, the effect was maintained during time, contrary to most disinfectants.
Discussion and Conclusions: The COVID-19 pandemic has had an immense impact on infection prevention and control methods, and disinfection interventions in the healthcare settings have been implemented. However, SARS-CoV-2 is indeed detected in the hospital environment, and massive use of disinfectants might increase AMR pathogens. Based on our results, PBS could respond to the urgent need of systems able to stably decontaminating from SARS-Cov-2 without worsening AMR concern, preventing the effects of an eventual future pandemics of secondary bacterial AMR infections.

81 - The glucocorticoid PYED-1 disrupts mature biofilms and inhibits virulence-factors in Candida spp

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Introduction

Candidemia and invasive candidiasis are severe healthcare associated infection associated to a high mortality rate and limited therapeutic options. *C. albicans* was the most prevalent cause of invasive fungal infections, and one of its most important virulence factors is the ability to form biofilms, becoming less susceptible to antifungal drugs. The increase of fungal species resistance to antifungal drugs led to a continuous need for the development of novel antifungal agents with broad spectrum, low toxicity and effectiveness against *Candida* species. This study aimed to assess the antifungal and anti-biofilm effects of the heterocyclic corticosteroid PYED-1 (pregnadiene-11-hydroxy-16alpha,17alpha-epoxy-3,20-dione-1) against *Candida* species.

Materials and Methods

Antifungal activity was determined by broth microdilution and calculation of colony forming unit, while the activity of PYED-1 against fungal biofilms was tested by crystal violet and tetrazolium salt reduction assay. The combination effects between PYED-1 and posaconazole or fluconazole or resveratrol against *C. albicans* cells were assessed by a microbroth checkerboard assay. The effect of PYED-1 on the germ tube formation was studied using a serum stimulation method.

Results

PYED-1 exhibited antifungal activity against *C. albicans* with minimum inhibitory concentration values of 32–64 μg/mL and against 4 other Candida species (*C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*). No synergism with fluconazole or posaconazole was observed; in contrast the association of PYED-1 with resveratrol has synergistic anticandidal activity, enhancing the PYED-1 susceptibility of fluconazole-resistant isolates. A promising alternative strategy to treat infections caused by multidrug pathogens is the development of drugs able to specifically inhibit virulence factors. PYED-1 inhibited the dimorphic yeast-to-hyphae morphogenetic transition of *C. albicans*, an important virulence mechanism of *C. albicans*. Moreover, PYED-1 used alone was able to eradicate *Candida* biofilms, another crucial virulence factor correlated with invasive fungal infection.

Discussion and Conclusion

The present results indicate that PYED-1 has the potential to serve as an anti-*Candida* treatment and preventive tool which functions by inhibiting existing or under-forming *C. albicans* biofilms.

84 - Graphene oxide a promising nanomaterial to structure innovative treatments against Mycobacterium tuberculosis infection.

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Introduction: Tuberculosis remains one of the most alarming infectious diseases worldwide, primarily affecting low-income countries, where the infection shows a higher and unvaried prevalence.
The emergence and spread of resistant *Mycobacterium tuberculosis* (*Mtb*) strains are causes of major concern, drawing attention to the development of new drugs and drug-repurposing strategies.
Among innovative approaches, promising results have been obtained against TB using carbon nanomaterials, especially against antibiotic resistant *Mtb* strains by shortening treatment periods, reducing adverse effects and mitigating antibiotic use.
Graphene oxide (GO) is a pioneering bi-dimensional nanomaterial with both drug loading capacity and antibacterial properties. When administered in vivo accumulates in the lungs and is readily degraded by macrophagic peroxidases.
Materials and methods: We evaluated GO anti-mycobacterial properties against *Mycobacterium smegmatis* (*Ms*) and *Mtb*. GO was able to trap mycobacteria in a dose-dependent manner, preventing the entry of bacilli into macrophages. However, GO did not show any anti-mycobacterial activity when used to treat infected cells or when macrophages were pre-treated before infection. We then tried to improve anti-mycobacterial effects of existing compounds. Particularly, our work explores how combinations of isoniazid (INH), amikacin (AMK) and linezolid (LZD) with GO impacts bacterial killing. Anti-mycobacterial properties were evaluated against *Mtb* using a checkerboard assay or an in vitro infection model.
Results: Different GO effects were observed when incubated with INH, AMK or LZD. Whereas the INH and AMK anti-mycobacterial activities were blocked by GO co-administration, LZD bactericidal effect increased in combination with GO. GO-LZD significantly reduced extracellular mycobacteria during infection and was able to kill internalized bacilli, likely due to increased production of ROS species.
Discussion and Conclusions: GO alone and GO-LZD co-administration appears to be a potential new promising anti-TB treatment at the forefront in fighting emerging antibiotic-resistant *Mtb* strains. This innovative pharmacological approach may lead to reduced treatment length and decreased adverse effects. More importantly, this work may draw attention to the use of nanomaterials-drugs combinations as possible strategy to quickly design drugs for infectious diseases' treatment.

32 - Cefiderocol: evaluation of in vitro antimicrobial activity in gram-negative bacteria

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1. Introductions

The spread of Multidrugresistant (MDR) and Extensivelydrugresistance (XDR) pathogens poses serious public health problems due to limited treatment options. In recent years, several molecules were been approved for clinical use in relation to encouraging effects in vitro and in vivo. Cefiderocol (CFDC; S-6492266) is a new siderophore-cephalosporin that acts on Gram negatives in particular on strains with a resistance to carbapenems. Structurally it shows a similarity to ceftazidime and cefepime, but the addition of a chlorocycle allows it to chelate iron imitating the action of siderophores present in nature. In this way, it is actively absorbed and has a more effective penetration overcoming some intrinsic or acquired mechanisms of antibiotic resistance.

2. Materials and Methods

We evaluated *in vitro* activity of the cefiderocol on clinical isolates Gram negative and Gram-positive multisensibili (MS), MDR and XDR, through two methods for the determination of the Minimum Inibente Concentration (MIC), microdilution in broth and "disk diffusion". The study covered 75 clinical isolates, 21 *A. baumannii* MS and 5 *A.baumannii* PDR (Pan Drugs Resistant) 14 *P.aeruginosa* MS and 8 *P.aeruginosa* MDR, 2 *K.pneumoniae* MS and 10 *K.pneumoniae* MDR, 5 *E.coli* MS and 4 *E.coli* MDR and 6 *S.aureus* MRSA. EUCAST MIC breakpoints had used to determine the sensitivity of different clinical isolates to the molecule.

3. Results

The determination of cefiderocol MIC sensitivity, by microdilution method in broth and "disk-diffusion" showed *in vitro* antimicrobial activity in most of Gram-negative strains and confirm inactivity towards *S.aureus* MRSA. In *K. pneumoniae* MS strains, cefiderocol showing significant activity. On the other hand, activity was absent in *K. pneumoniae* MDR. In *A. baumannii* with colistine sensitivity profile (COS strains), the cefiderocol was sensitive. On the contrary, in colistine-resistant strains (COR) was resistant. Finally, cefiderocol had effective action in *P.aeruginosa* MS and MDR. The value of the MIC/breakpoint ratio (RMB) suggests, in some MDR strains, a possible use of cefiderocol in combination with other antibiotics. The association tests in *K. pneumoniae* MDR strains showed additive effect of cefiderocol/meropenem as same as ceftazidime-avibactam/colistine combination. In *A. baumanni* PDR/COR, cefiderocol was synergistic with colistin, ampicillin-sulbactam and phosphomycin. Finally, the combinations tested on *P. aeruginosa* MDR strains showed a synergy of cefiderocol with meropenem and phosphomycin.

4. Discussion and Conclusions

Cefiderocol showed a good antibacterial action *in vitro* against most of the Gram negative MS, MDR and XDR tested. The use of microdilution is preferable. Cefiderocol represents a valid alternative in infections complicated by multiresistant Gram-negative bacteria mainly on *P.aeruginosa*. In *A.baumannii* COR and *K.pneumoniae* MDR, the MIC obtained showed an action compared to the main antimicrobial agents. Cefiderocol have promising use in association with other antimicrobial molecules. In *P.aeruginosa* with phosphomycin than in *A.baumannii* PDR/COR the antibiotics

association of cefiderocol and ampicillin sulbactam are comparable. The reduced efficacy in *K.pneumonia* KPC confirmed the efficacy in monotherapy and in association.

105 - New promising strategy to tackle the Helicobacter pylori resistance: the role of Resveratrol derivatives

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Abstract

Introduction. The multidrug resistance in *Helicobacter pylori* represents an alarming phenomenon that underlines the stringent need of novel strategies to improve the eradication rate. There is a great attention in alternative treatments combining antibiotic and non-antibiotic compounds, such as Antibiotic Resistance Breakers, expressing antibacterial/anti-virulence activities restoring the efficacy of conventional drugs. Over recent years, resveratrol (RSV) attracted great attention for its multifaceted biological activities like anti-inflammatory, anti-carcinogenesis, and anti-aging, including antimicrobial activity, although the *in vivo* application is limited for its poor bioavailability.

Materials and Methods. In this study, the antibacterial (MIC/MBC) and anti-virulence effects (biofilm reduction and swarming motility inhibition) of RSV and new synthetized RSV-phenol derivatives, with a higher bioavailability, alone and combined with levofloxacin-LVX were evaluated against resistant *H. pylori* clinical strains. The experiments were confirmed *in vivo* using the *Galleria mellonella* model.

Results. The results showed an increased antibacterial action of the new RSV derivatives, RSV-3 and RSV-4, with lower MIC (6.25 - 200 □g/ml and 3.12 - 200 □g/ml, respectively) in respect to the RSV (MIC= 400-800 □g/ml). RSV, RSV-3, and RSV-4 were able to synergize with LVX restoring its effect in two out of seven clinical resistant strains tested for the study. RSV, and RSV derivatives, alone and combined with LVX at sub-MIC and sub-synergistic concentrations, significantly reduced the biofilm formation. Moreover, RSV-3 and RSV-4 reduced the swarming motility of *H. pylori* on soft agar. *In vivo* studies showed that RSV, RSV-3, and RSV-4 were non-toxic for *G. mellonella* larvae displaying a protective effect against *H. pylori* infection.

Conclusion. Overall, the results underline the anti-*H. pylori* effect of RSV-derivatives, representing interesting candidates for innovative therapeutic schemes to tackle the *H. pylori* antibiotic resistance.

This work was funded by a Research Grant PRIN 2017 SFBFER from MIUR, Italy.

100 - Sardinian Foeniculum vulgare Miller essential oil shows promising antifungal activity in vitro: a preliminary characterization

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Vulvovaginal candidiasis (VVC) is a common distressing disease, with three-quarters of women affected during their lifetimes. Due to the increasing incidence of both fungal infections and antifungal resistance to common antimicrobial drugs, the need to find alternative therapies is growing. In this context, plant derived products such as essential oils (EOs) are a promising source of compounds with antifungal activity. This study aimed investigating the application of *Foeniculum vulgare* Mill. EO as a potential innovative antimicrobial agent against VVC.

Materials and Methods

F. vulgare Mill. essential oil (EO) was obtained via standardized extraction methods, and its chemical composition was determined by gas chromatography. The EO antifungal properties were investigated in vitro against 5 *Candida* species: *Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis* and *Candida parapsilosis* collected at the Laboratory of Diagnostic Microbiology (Azienda Ospedaliera Universitaria -AOU, Sassari). We evaluated the value of the minimum fungicidal concentration (MFC) of EO through the broth microdilution method, using concentrations ranging from 2% to 0,01 % (v/v). All *Candida* strains were also tested for sensitivity to Amphotericin B. EO activity was evaluated also against *Lactobacillus acidophilus* using the same dilutions described above.

Results

C. krusei and *C. tropicalis* strains tested were resistant to Amphotericin B. Data analysis showed that *F. vulgare* Mill. EO had an effective, dose-dependent fungicidal activity against all *Candida* species tested. At the same time, this EO showed no toxic activity towards *L. acidophilus* even at the highest concentrations used.

Discussion and conclusion

EOs are known for their antimicrobial properties, and their use in the treatment of VVC is growing also in order to counter the increasing resistance to common antifungal drugs. The aim of this study was to find an alternative for antifungal drugs currently used in the treatment of VVC. Our results showed a marked i*n vitro* antifungal activity, suggesting a promising potential application of *F. vulgare* Mill. EO in the treatment of VVC. Furthermore, *F. vulgare* EO is not toxic against *L. acidophilus*, whose presence in the vaginal milieu represents a crucial protective factor against pathogen proliferation. The results of this work encourage the search for new alternatives or complementary therapies against VVC.

112 - Citrus bergamia: Kinetic of antimicrobial activity on clinical isolates

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Introduction: The inappropriate use of antibiotics has increased selective pressure and spread of Multi-Drug Resistant (MDR) pathogens, which reduces the possibility of effective treatment. A potential alternative therapeutic approach may be represented by essential oils, as the distilled extract of bergamot. The aim of this work was to evaluate the kinetic of bactericidal and fungicidal activity of the distilled extract of bergamot on MDR bacteria and fungi of clinical isolates using the time-kill assay. Furthermore, the antimicrobial activity of the distilled extract of bergamot on the morphology and cellular organization of clinical pathogens was evaluated by Confocal Laser Scanning Microscopy (CLSM) to address the possible antimicrobial mechanisms.
Materials and Methods: MDR bacteria and fungi were isolated from patients admitted to the Catanzaro University Hospital (Italy). For the determination of the Minimal Bactericidal (or Fungicidal) Concentration (MBC or MFC), serial dilutions of distilled extract of bergamot (from 5% to 0.03% *v/v*) were prepared. To evaluate killing kinetics, time kill assay was carried out. Finally, to determine the viability of MDR bacteria after 4 hours treatment with distilled extract, each preparation was labeled with acridine orange stain and observed with CLSM. Instead, the yeast cells were stained with DAPI to evaluate nuclear modifications and with Calcofluor White and Concanavalin A to evaluate cell membrane morphology (chitin and α-mannopyranosys layer, respectively) before, during and after treatment with the distilled bergamot extract.
Results: Distilled extract of bergamot has a slow bactericidal activity on Gram-positive bacteria, while bactericidal effect on Gram-negative bacteria was observed after 2 hours of exposure. Finally, on the *Candida* strains the fungicidal effect was confirmed after 30 minutes of exposure. In addition, the Confocal Microscope images confirmed the death of almost all bacterial cells after 4 hours of treatment. On the contrary, after one hour of treatment for fungi, the rounded structure of nucleus is no longer observable, the chitin layer seems to gradually decrease while the mannan layer remains stable.
Discussion and Conclusions: Antimicrobial activity of distilled extract of bergamot on MDR clinical isolates was confirmed with a greater sensitivity of Gram-negative strains compared to Gram-positive. The prompt fungicidal activity on *Candida* strains can be accounted by the presence of sterols on the yeast cell wall, allowing a faster interaction with the lipophilic components of bergamot. This study confirms the usefulness of molecules of natural origin as a valid alternative to common antibiotic therapy or as adjuvants in contrasting drug resistance mechanisms.

82 - Effects on cell viability, growth, and morphology of Candida spp. biofilms after synergistic treatment with the human lactoferricin derived peptide hLF1-11 and Caspofungin

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Introduction. *An important Candida virulence factor is the ability to grow as biofilm, consisting in a three-dimensional structure and different cell types, e.g., yeast cell, hyphae and pseudohyphae embedded in a cellular matrix. Biofilm infections are often associated with increased morbidity and mortality, and often difficult to treat.*
As new strategy in biofilm therapy, antimicrobial peptides, alone or in combination with conventional drugs, represent a good opportunity employed recently.
*The present study was aimed at investigating the effect of the synergistic activity of the N-terminal lactoferrin-derived synthetic peptide (*hLF1–11) and caspofungin *against biofilm-induced by different strains of C. albicans* and *C. parapsilosis* by Operetta High-Content Imager (PerkinElmer).
Materials and Methods. Biofilm-induced by *C. albicans* SC5314, *C. albicans* CACR (caspofungin resistant strain) or *C. parapsilosis* clinical isolate (CP7, selected for its strong ability to produce biofilm) was tested in 96-well plate with a glass bottom. Strains were treated both with hLF1–11 and caspofungin at Minimal Inhibitory Concentrations (MIC) and with drug concentrations identified as synergistic in a previous checkerboard assay.
Live/dead staining was performed by combining the green-fluorescent nucleic acid stain SYTO 9 and the red-fluorescent nucleic acid stain propidium iodide in PBS. Epifluorescence images were acquired using the Operetta CLS High-Content Imager and the Harmony software.
Results. The positive control (untreated) shows a thick multilayer biofilm rich in hyphae or pseudohyphae, positive to SYTO 9 staining, for all the tested strains. The synergistic combinations of hLF1-11 and caspofungin show the reduction of cells positive to SYTO 9 and the presence of many dead cells stained with propidium iodide, like the effect obtained by the MIC concentration of each drug alone. In *C. albicans* the synergistic combinations cause a complete inhibition of hyphal production (0.25 mg/ml caspofungin + 5.5 mg/L hLF1-11 for CACR and 0.06 mg/ml caspofungin + 5.5 mg/L hLF1-11 for SC5314), whereas the same concentration of each drug shows a low antibiofilm activity. In *C. parapsilosis* the synergistic effect is obtained with 0.25 µg/mL caspofungin + 11 mg/L hLF1-11.
Discussion and Conclusions. Fluorescence imaging analysis confirmed the synergistic effect observed between caspofungin and hLF1-11 against biofilm-induced by *Candida species* previously evaluated by metabolic and cell viability assays. Antimicrobial peptide alone or in combination with conventional antifungal drug represents a possible interesting novel approach to treat or prevent fungal biofilm infections.

Acknowledgements: We thank Merck Sharp & Dohme Corp. for providing part of the study material.

83 - Epidemiology and Pattern of Resistance of Gram-Negative Strains Isolated from Blood Samples in Hospitalized Patients: A Retrospective Analysis.

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Introduction: Blood culture is considered the mainstream tool to detect microorganisms when a hospital acquired blood stream infection is suspected and for the diagnosis of any bacteremia. Mortality due to bacteremia is related to the type of organism isolated and the nature of any underlying condition. Early positive results allow the targeted antimicrobial treatment with a significant benefit in reducing mortality.
Materials and methods: A retrospective analysis on blood sample positive for bacteria of Gram-negative strains were collected from patients hospitalized from more than 48 hours at Sant' Elia hospital, Caltanissetta, Italy from January 2018 to April 2020. We divided the patients 'age range in four equal intervals. The relationships between mortality and demographic, and microbiological variables were analyzed.
Results: Bacteria distributions in positive blood cultures is not associated neither to gender nor to the age. In other words, any bacteria isolated in our patients has not a significant difference as far as male and female and among age groups. In addition, we can observe that in the male group the most frequent Gram-negative strains were *Klebsiella pneumoniae* and *Acinetobacter baumanii* otherwise in the female group the most frequent strains were *K. pneumoniae* and *Escheria coli*. Similarly, results were obtained considering the variable age and considering for it four equal intervals. The analysis of strains by no Intensive Care Unit (no ICU) and Intensive Care Unit (ICU) ward showed a different pattern of Gram-negative strains with prevalence of *K. pneumoniae* and *A. baumannii* in ICU and *E. coli* in no ICU wards. Regarding the analysis of single antibiotic resistance, the strains of *E. coli* were susceptibility to a large classis of antibiotic as carbapenem and trimethoprim–sulfamethoxazole, while *K. pneumoniae* showed a significant susceptibility to colistin, tigecycline and trimethoprim–sulfamethoxazole. From survival analysis, it resulted a significant difference between survival time of patients in ICU and no ICU. In addition, patients with *E. coli* had more survival rate in comparison to patients with different bacteria.
Discussion and Conclusions: The prevalence of *E. coli* in no ICU setting especially in female and geriatric patients encourage once again the implementation of community-level programs to prevent gram negative bacteremia and urinary tract infection. The *E. coli* survival analysis and *K. pneumoniae* and *E. coli* susceptibility pattern to some antibiotics included in prescription patterns of general practitioners suggests that local surveillance and implementation of educational programs remain the essential measures to slow down the spread of resistance and consequently increase antibiotic life span.

150 - Outer membrane vesicles are a driving force for horizontal gene transfer

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Introduction: Horizontal gene transfer (HGT) is the main source of genetic material transfer between microorganisms. It contributes to bacterial evolution, increasing bacterial survival, adaptation in hostile environments and pathogenicity. Recent studies identify OMVs as a new mechanism of HGT, however the parameters that mediate this process remain unclear. The current study shows, for the first time, the transfer of plasmids containing beta-lactamase resistance genes via OMVs derived from *Klebsiella pneumoniae* (*K. pneumoniae*).

Materials and Methods: *K. pneumoniae* ATCC was transformed through the calcium chloride method with pGR (high copy number) and PRM (low copy number) plasmids. OMVs were purified from *K. pneumoniae*-pGR and -PRM and characterized via transmission electron microscopy (TEM), Dynamic Light Scattering (DLS) and mass spectrometry. The plasmid concentration in the vesicular lumen was determined by Real-time PCR. For gene transfer experiments, *K. pneumoniae*, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (P. *aeruginosa*), *Burkholderia cepacia* (*B. cepacia*) and *Salmonella enterica* (*S. enterica*) ATCC were used as recipient strains. These strains (10^7 CFU/mL) were incubated with 10 µg of OMV-*K. pneumoniae* for 8 hours at 37 °C and after plated on LB agar ampicillin. The bacterial colonies grown were counted for the transformation efficiency and subjected to colony-PCR for detection of the resistance genes. Bacterial susceptibility to ß-lactams (ampicillin and piperacillin), before and after OMV treatment, was assessed via disk diffusion assays.

Results: OMVs appeared on TEM as electron-dense particles with spherical morphology. DLS data showed that OMVs derived from *K. pneumoniae*-pGR had a diameter of 113.8±53.7 nm, while *K. pneumoniae*-PRM measured a size of 94.13±41.10 nm. Mass spectra analysis detected 14 membrane-associated proteins, 3 periplasmic proteins, 32 cytosolic proteins and 21 enzymes. OMVs from *K. pneumoniae*-pGR contained 10.4±0.05 ng DNA/µg OMV, while *K. pneumoniae*-PRM had 08±0.62 ng DNA/µg OMV. Vesicles from *K. pneumoniae*-pGR and -PRM induced a transformation efficiency of $2.8\pm0.1\times10^4$ CFU/µg and $7.8\pm0.9\times10^3$ CFU/µg, respectively. OMVs derived from *K. pneumoniae*-pGR transferred DNA to *E. coli*, *S. enterica*, *P. aeruginosa* and *B. cepacia* with a transformation efficiency of $1.7\pm0.2\times10^4$, $1.5\pm0.9\times10^4$, $1.6\pm0.1\times10^4$, $1.8\pm0.8\times10^4$ CFU/µg, respectively. After the OMV-HGT, no inhibition area was detected for β-lactam drugs, proving the acquisition of resistance.

Discussion and Conclusions: This study proved the spread of beta-lactamase resistance via OMV-*K. pneumoniae*. This novel HGT mechanism was dependent on the identity of the genetic cargo and not related to the phylogenetic characteristics of the donor and the recipient species.

26 - The mef(A)/msr(D)-carrying prophage phi1207.3 encodes an SOS-like system responsible for both increased survival and antibiotic resistance mutation rates in Streptococcus pneumoniae

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Introduction

Unlike other streptococci, *Streptococcus pneumoniae* lacks a chromosomally encoded SOS system, but SOS response genes can be introduced by mobile genetic elements. Phi1207.3 is a functional prophage carrying the *mef*(A)/*msr*(D) macrolide efflux genes, and also an SOS-like cassette, responsible for SOS-induced mutagenesis in *Streptococcus uberis*. In this study, we investigated if phi1207.3 presence leads to an SOS-like response activation upon exposure to chemical and physical stresses in *S. pneumoniae*.

Materials and Methods

S. pneumoniae recombinant strains were produced by gene SOEing PCR and transformation. Mitomycin C and UV-C light were used as chemical and physical stresses. For UV-C light, a holder and autoclavable plates system was designed and 3D-printed to be used with a LED instrument, allowing monochromatic (265nm) irradiation. UV-C light survival was assessed by plating and calculated as a ratio between cell viability of irradiated and not irradiated cultures. UV- and mitomycin C-induced mutagenesis was assessed by measuring the mutation rate through fluctuation assay, inoculating 30 independent cultures with 10^5 CFUs of pneumococci, respectively irradiated with 50 J/m^2 UV-C fluence, and treated with 100 ng/ml mitomycin C. Upon reaching mid-log phase, number of rifampicin- and optochin-resistant mutants was assessed by plating. Mutation rates determination and statistical analysis were carried out with R package rSalvador.

Results

A group of 3 isogenic strains, i) devoid of phi1207.3, ii) carrying phi1207.3, iii) carrying phi1207.3ΔSOS-like cassette, were constructed in both unencapsulated *S. pneumoniae* strains R6 (*hex+*) and Rx1 (*hex-*, deficient for Mismatch Repair System, displaying higher mutation rates). For both groups, strains carrying phi1207.3, containing the SOS-like cassette, showed a significant increase in UV-C light survival, compared to the control strains devoid of phi1207.3, and carrying the recombinant phi1207.3 with the SOS-like cassette deleted. Phi1207.3-carrying strains also showed a significant increase in the mutation rate upon exposure to UV-C light and mitomycin C, whereas no significant increase was observed in absence of the SOS-like cassette.

Discussion and conclusions

The *mef*(A)/*msr*(D)-carrying phi1207.3 is the first example of a functional prophage harboring an antibiotic resistance determinant able to transfer among streptococci. We demonstrated that phi1207.3 also carries a functional SOS-like cassette, responsible for an SOS-like response in *S. pneumoniae*. Hence, phi1207.3 presence not only leads to drug resistance but is also responsible for

increased bacterial survival and higher probability of acquiring mutations in response to both chemical and physical stresses.

155 - Evaluation of antibiotic resistance and biofilm production among clinical strain isolated from medical devices.

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Introduction Microbial biofilms pose a serious threat to patients requiring medical devices (MDs). Prolonged periods of implantation carry a high risk of device-related infections (DRIs). Patients with DRIs often have negative outcomes following the failure of antibiotic treatment. Resistant DRIs are mainly due to the MDs contamination by bacteria producing biofilm. The present study aimed to detect biofilm formation among MD bacterial isolates and to explore their antibiotic resistance profile.

Materials and Methods The study was conducted on 76 MDs, collected at university Hospital of Campania "Luigi vanvitelli", between October 2019 and September 2020. Identification of isolates and antibiotic susceptibility testing were performed using matrix assisted laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) and Phoenix Becton Dickinson, respectively. Biofilm-forming abilities were assessed usaed the crystal violet staining method. **Results** Among the 94 MDs isolated strains, 42.7% were Gram-positive, 40.3% Gram-negative and 17% Candida species. Among 78 bacterial strains, 43,6% were non-limited spectrum of antibiotic classes. All moderate and strong biofilm producers and 81% of weak biofilm producers were Multi Drug resistance (MDR) strains. In contrast, among non-biofilm producers, only 11.8% were classified as MDR strains.

Conclusions Sulfamides for Gram-positive strains and Fluoroquinolones, Carbapenems and Aminoglycosides for Gram-negative isolates were found to be more effective for most biofilm producing strains. To better understanding biofilm-producing strains isolated from MDs and related antibiotic resistance profiles could help define a more effective treatment plan to improve patient management and stimulate the scientific community to search for new treatment strategies to combat this real threat

62 - Potential activity of Albino Grifola frondosa extract against biofilm of Listeria monocytogenes

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Introduction: *Listeria monocytogenes* is a Gram-positive microrganism with natural habitat in soils, plants and water. Living on the ground and in surface waters, it is with the raw materials that Listeria enters the food industries. This bacterium can have very serious consequences on humans causing listeriosis if ingested in large concentrations through contaminated food. In recent years, it has been made known that Listeria is not only capable of living within biofilms formed by other microorganisms but is itself a biofilm promoter. Protected by biofilm, it becomes not very sensitive to disinfection treatments and multiplies undisturbed continuing to contaminate food and drinks with which it comes into contact. For these reasons, we are looking for natural substances that can have an antibiofilm action. Albino *Grifola frondosa* (Maitake in Japanese language) is a mushroom mainly distributed in Asian countries. As well as being edible, it is acknowledged also for its inhibiting action on bacteria. Therefore, the aim of the study was to test the potential antibiofilm effect of mushroom extracts on clinical strains of *L. monocytogenes.*
Materials and Methods: The assay for biofilm formation and evualuation of the inhibitory activity of *G. frondosa* extracts for the clinical isolates of *L. monocytogenes*, received from patients at Palermo hospitals, were performed in triplicate with microplate, using the crystal-violet method.
Results: According to our tested conditions, only one strain was classified as a non-producer of biofilm, while 60% of strains showed a weak biofilm production and 34% of the strains were classified as a moderate biofilm producers. The activity of *G. frondosa* mushroom extracts show a statistically reduction of OD value measured for all strains examined, associated with a strong reduction of biofilm in terms of biomass. The percentage reduction in OD for the treated strains compared to untrated strains is on average 91%, for 40% of the strains classified as moderate biofilm producers were about 100%.
Discussion and Conclusions: Also in our study, the results of the biofilm formation assay show that the analized strains of *L. monocytogenes* are capable of producing biofilm. The activity of *G. frondosa* mushroom extracts highlight a significant reduction in OD between untreated and treated isolates producers of biofilm. The preliminary results obtained encourage the holding of new ones essays to evaluate the potential of *G. frondo*sa mushroom extracts. It would be desirable to use them both as a preventive treatment to inhibit the formation of biofilm on the surface of the containers used for food storage and on the surface of materials used in the industrial field and on already formed biofilms.

14 - A simple test for weekly detection of Mycoplasma contamination in cell cultures

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Mollicutes (*Mycoplasma* and *Acholeplasma*) are parasitic bacteria that adhere to cellular surfaces, naturally resistant to many antibiotics and extremely small. They are often found as contaminants in cultured cells, where they go unnoticed. They may be present in viral stocks because they are present in supernatants of cells where cultured viruses are released. The best way to keep laboratories free of *Mycoplasma* is to discard infected cultures, but, as judged by the very common finding of *Mycoplasma*-contaminated cultures in many laboratories, this is not done as often as it should be. A possible reason is that most procedures recommended take as long as performing a simple experiment and many laboratories delay testing to save money and time. Indeed, many methods exist to detect *Mycoplasma* infection of cell lines, but they take at least a couple of hours of hands-on work, if not more.

Here we describe our experience with a procedure to screen viral stocks and tissue cultures for *Mycoplasma* presence. The procedure relies on isolation of *Mycoplasma* on ordinary horse blood agar directly from exhausted tissue culture supernatants and does not require experienced personnel or expensive equipment. It only requires minutes of hands-on work and, for this, it may be useful for weekly screening of cultures. It yields semiquantitative results in roughly 5 days, which is the time that usually passes between one subculture passage of cells *in vitro* to another. Because of its simplicity, it may be useful for detecting *Mycoplasma* in viral stocks and for frequent screening of cultures in research laboratories.

We tested our method on a number of cell culture supernatants from 11 different laboratories and on viral stocks with intriguing results.

159 - Thiazolides inhibit canine coronavirus replication at a post-entry level

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Introduction: The *Coronaviridae* family comprises a large number of enveloped, positive-sense single-stranded RNA viruses causing respiratory, enteric and neurological diseases of varying severity in domestic and wild animals, as well as in humans. There is no effective antiviral chemotherapy for most coronaviruses (CoV). The thiazolides nitazoxanide (NTZ) and tizoxanide (TIZ) have been shown to possess antiviral activity against several RNA viruses, including coronaviruses; however the mechanism of the anti-CoV activity remains to be elucidated. Herein we investigated the effect of thiazolides on coronavirus replication, using a canine coronavirus (CCoV) as a model. ***Materials and methods:*** Canine adenocarcinoma (A72) cells were infected with CCoV (strain S-378) under single-step and multi-step growth conditions. NTZ and TIZ were dissolved in DMSO and diluted in culture medium before treatment. Virus yield was determined by $TCID_{50}$ infectivity assay, and cell viability was determined by MTT assay. Viral proteins were characterized by SDS/PAGE-autoradiography after [^{35}S]-methionine/cysteine-labeling and by Western-blot analysis. CCoV genomic RNA levels were determined by real-time PCR. ***Results:*** NTZ, and its active metabolite TIZ, are effective in inhibiting CCoV replication at low micromolar concentrations; thiazolide treatment also protects the host cell by the virus-induced cytopathic effect. Thiazolides do not affect virus adsorption or entry into the host cell. Analysis of CCoV protein synthesis by Western blot and [^{35}S]-methionine/cysteine-labeling demonstrate that thiazolide treatment started after viral entry potently inhibits CCoV protein expression and prevents the virus-induced shut-off of the host cell protein synthesis up to 24 h p.i.. Thiazolides also potently inhibit the CCoV RNA synthesis up to 24 h p.i.. ***Discussion and Conclusions:*** Nitazoxanide is used in the clinic as a safe and effective antiprotozoal/antimicrobial drug; its antiviral activity was shown in patients infected with hepatitis-C virus, rotavirus and influenza viruses, and, more recently, in COVID-19 patients. Using a canine coronavirus as a model, we now show that nitazoxanide does not affect virus binding or entry, but acts at a post-entry level, causing a block of viral RNA and protein expression, and protecting the host cell against the virus-induced damage

46 - Evaluation of isavuconazole in vitro activity against Candida species: comparison between MIC Strip and EUCAST reference method

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Introduction: Isavuconazole is a new triazole with an expanded-spectrum and a potent activity against moulds and yeasts. In vitro, isavuconazole is highly active against bloodstream isolates of *Candida* spp. and it has demonstrated good efficacy in animal models of candidiasis. Unfortunately, EUCAST epidemiological cutoff values and breakpoints for *Candida* spp. have not been established yet, due to a significant interlaboratory variability. The only commercially available isavuconazole susceptibility test is the MIC strip test. This retrospective study aims to assess the isavuconazole *in vitro* activity comparing gradient minimum inhibitory concentration (MIC) strips with the EUCAST broth microdilution reference method.

Materials and methods: A total of 216 *Candida* strains of several *Candida* sp. were envolved in the study design: 111 strains of *C. albicans*, 9 strains of *C. krusei*, 13 strains of *C. glabrata*, 28 strains of *C. tropicalis*, 52 strains of *C. parapsilosis* complex, 1 strain of *C. famata*, 1 strain of *C. guillermondii* and 1 strain of *C. incospicua* were recruited. All the strains were isolated from blood samples of patients admitted to A.O.U. "G. Rodolico - San Marco" Hospital, whose samples were collected from January 2015 to December 2019. Isavuconazole susceptibility for all the isolates was tested using both the EUCAST broth microdilution method (E. Def 7.3.1) and the MIC strip isavuconazole test (Liofilchem, Roseto degli Abruzzi, TE, Italy).

Results: The geometric means for the MICs using the EUCAST reference methods and the MIC strip test were respectively: 0.013 mg/l and 0.019 mg/l for *C. albicans*, 1.615 mg/l and 1.545 mg/l for *C. glabrata*, 0.734 mg/l and 0.564 mg/l for *C. krusei*, 0.015 mg/l and 0.013 mg/l for *C. tropicalis*, 0.011 mg/l and 0.016 mg/l for *C. parapsilosis* complex. The modal MICs using the EUCAST reference methods, and the MIC strip test were respectively: 0.008 mg/l and 0.016 mg/l for *C. albicans*, 2 mg/l and 2 mg/l for *C. glabrata*, 0.5 mg/l and 0.5 mg/l for *C. krusei*, 0.008 mg/l and 0.004 mg/l for *C. tropicalis*, 0.008 mg/l and 0.032 mg/l for *C. parapsilosis* complex. The essential agreement between the isavuconazole MIC strips and the EUCAST reference method was 83.7%.

Discussion and conclusions: This study describes the *in vitro* activity of isavuconazole using gradient concentration MIC strips, compared with the EUCAST broth microdilution reference method. According to our results, isavuconazole seems to have good *in vitro* activity against *Candida* isolates. Further studies are needed to increase the isavuconazole *in vitro* data and to define clinical breakpoints for all *Candida* spp.

157 - Repurposing apramycin and ribavirin against Pseudomonas aeruginosa causing lung infections in cystic fibrosis patients

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Introduction. Chronic lung infections are recurrent in cystic fibrosis (CF) patients, and *Pseudomonas aeruginosa* is the main pathogen involved. These infections cannot be eradicated due to *P. aeruginosa* antibiotic resistance which is further aggravated by its ability to grow as biofilm. Thus, there is an urgent need to develop new antibiotic molecules. The "drug repurposing" strategy – i.e., the finding of novel therapeutic indications for existing drugs - might be a promising tool for new antimicrobials discovery. In this study, nine FDA-approved drugs were tested, comparatively with tobramycin (TOB), for *in vitro* activity against planktonic and biofilm cells of *P. aeruginosa* from CF patients. **Materials and Methods**. Two *P. aeruginosa* isolates (Pa7 TOB-S, and PaPh32 TOB-R) from CF patients, selected because strong biofilm producers and multi-drug resistant (MDR), were tested. The minimum inhibitory and bactericidal concentrations (MIC, MBC) of TOB and ribavirin (RIB; antiviral), toremifene (TOR; nonsteroidal antiestrogen), oxyclozanide (OXY; anthelmintic), meloxicam (MEL; nonsteroidal anti-inflammatory drug), apramycin (APR; veterinary aminoglycoside), 5-fluorouracil and actinomycin D (5-FLU and ACT; antineoplastics), furosemide (FUR; diuretic) and ciclopirox (CIC; antifungal) were evaluated by broth microdilution. The activity against preformed 24h-old biofilm was assayed by quantification of biomass (crystal violet assay) and residual viability (viable cell count). Finally, the cytotoxic effect of each drug was assessed towards IB3-1 bronchial epithelial cells. After 24h-exposure to each drug, cell viability was measured using MTS tetrazolium-based colorimetric assay. **Results**. TOB was the most active drug (MIC: 2 and 64 µg/ml; MBC: 4 and >64 µg/ml for Pa7 and PaPh32, respectively), followed by APR (MIC: 64 and 32 µg/ml; MBC: 256 and 64 µg/ml, respectively). ACT, CIC, and 5-FLU exhibited limited activity, while RIB, OXY, MEL, FUR and TOR showed any. Among the five active drugs, CIC was the most effective against biofilm, regardless of concentration and strain tested (biomass removal range: 75-80% and 69.5-83.5%, respectively for Pa7 and PaPh32). Among drugs not active against planktonic cells, RIB was the most effective in dispersing biofilm biomass (biomass removal: 66.8-38.1% at 1024 µg/ml, respectively for Pa7 and PaPh32). TOB, ACT, 5-FLU and RIB were not toxic for IB3-1 cells. Contrarily, the exposure to MEL, ACT, and CIC caused a significant reduction of cell survival.

Discussion and Conclusions. The antibacterial activity of APR and the efficacy of RIB against mature biofilm, along with their safety profile, make these drugs eligible for repurposing against *P. aeruginosa* infections in CF patients. Further *in vitro* and *in vivo* studies are warranted to confirm our preliminary results.

16 - Antimicrobial, antibiofilm and antivirulence activities of Diacerein and Rhein against Staphylococcus aureus and Pseudomonas aeruginosa.

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Introduction. Drug repositioning is an attractive and promising alternative in the treatment of infections caused by multidrug resistant microorganisms. The literature reports that many anti-inflammatory drugs possess antimicrobial activity. Diacerein (Dia) is an anti-inflammatory drug used for the management of osteoarthritis. Dia is scarcely stable in physiological conditions and it is rapidly hydrolyzed to its active metabolite rehin (Rhe). Previous studies have shown that Dia has antibacterial activity against *S. aureus* both *in vitro* and in an *in vivo* model of bacterial keratitis. The main aim of this study was to evaluate the antibiofilm and anti-virulence activity of Dia and Rhe against *P. aeruginosa* and *S. aureus. Materials and methods*. The susceptibility of bacterial strains to Dia and Rhe was evaluated by determination of MIC values and by bactericidal assays. The antibiofilm activity of both compounds was evaluated by cristal violet staining as ability to inhibit the biofilm formation. The anti-virulence effect of Dia and Rhe on *P. aeruginosa* was assessed by quantification of pyoverdine and proteolytic activity in supernatants of cultures exposed for 48h to both compounds. The anti-virulence activity of Dia and Rhe towards *S. aureus* is under investigation. Cytotoxicity evaluation was performed on murine fibroblasts by using WST-1 assay. Dia hydrolysis to Rhe was monitored at 37°C in PBS pH 7.4 by UV-VIS spectrometry at 368nm and 472nm, respectively. *Results*. Both Dia and Rhe showed a bacteriostatic effect against *S. aureus* whereas *P. aeruginosa* resulted insensitive. Rhe showed antibiofilm activity against *S. aureus* when tested at 12.5 □g/ml whereas the reduction of biofilm formation caused by Dia was not statistically significant. Both compounds did not affect the biofilm formation of *P. aeruginosa*. No evident anti-virulence effect of Dia and Rhe was also observed against *P. aeruginosa*. IC_{50} on murine fibroblasts resulted 116.2 µg/mL and Rhe 6.7 µg/mL for Dia and Rhe, respectively. In vitro hydrolysis of Dia to Rhe under simulated physiological conditions demonstrated that about 60% of Dia was converted to Rhe in 1h. *Discussion*. Dia and Rhe exert antibacterial effects against *S. aureus*, while they are inactive towards *P. aeruginosa* suggesting differences in the mechanism of antibacterial activity of both compounds against Gram-negative and Gram-positive species. Interestingly, Rhe shows a high degree of similarity to other anthraquinones with antibiofilm activity against *S. aureus* suggesting that the higher efficacy of Rhe than Dia in antibiofilm activity against *S. aureus* may be due its chemical structure. Ongoing studies will help to evaluate the therapeutic potential of Dia and Rhe as antimicrobial drugs against *S. aureus* infections.

15 - Extended Spectrum Beta Lactamases (ESBL)- and Carbapenemases-producing Enterobacterales from surface and groundwater in Lombardy, Italy

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Introduction: The study, supported by the Project "Circular Economy for Water and Energy" (CE4WE) call "Hub Research and innovation", aimed to screen for the presence of Extended-Spectrum Beta Lactamases (ESBLs), BL (AmpCs), and/or carbapenemases-producing *Enterobacterales* in surface and groundwater in Lombardy, Italy. **Materials and Methods:** on September 2020, n=30 water samples were collected from Vigevano (PV) and Abbiategrasso (MI) areas. A total of 50ml of water from n=23 fontanili, n=4 wastewater treatment plants (WWTPs), n=2 Ticino river stretches, and n=1 pond, was filtered using 0.45µm-pore size membranes. Filter membranes were then placed on Plate Count Agar (PCA), MacConkey Agar (MCA), and selective MCA (cefotaxime 0.5mg/L-2mg/L; meropenem 0.25mg/L-4mg/L). The total bacterial load/site was estimated after 24h incubation at 37°C. Species identification and antibiotic susceptibilities were obtained by MicroScan autoSCAN-4 System. Multiplexed-tandem RT-PCR (EasyPlexAssay, AusDiagnostic) allowed the BL genes detection; in multi drug resistant (MDR) strains, *aac(6')-Ib-cr* and *qnr*S genes were also identified by targeted PCR. **Results:** Gram-negatives resulted the prevalent bacterial population (PCA/MCA < 2) for 22/30 sampling sites, 8/30 and 6/30 of which showing high bacterial counts on cefotaxime and meropenem selective MCA, respectively. A total of n=18 *Klebsiella* spp. (n=11/18 *K. pneumoniae;* n=7/18 *K. oxytoca),* n=14 *Escherichia coli,* n=12 *Citrobacter* spp. (n=6/12 *C. freundii,* n=4/12 *C. brakii* and n=2/12 *C. farmerii*) n=2 *Enterobacter* spp. (n=1/2 *E. cloacae* and n=1/2 *E. hormaechei*), mainly from Abiategrasso area was isolated from selective MCA. Third generation cephalosporins (3GCs)-, aminoglycosides-, carbapenems-, and floroquinolones (FQs)- resistance was found in 6/18 and 1/18 *Klebsiella* spp. isolates, respectively. A total of 4/18 *Klebsiella* spp. strains resulted *bla*CTX-M-1, 2/18 *bla*TEM-type and 1/18 *bla*OXA-1 genes positive by RT-PCR. A MDR *K. pneumoniae* strain collected from a WWTP of MI area was *bla*KPC-type genes positive. Moreover, n=7 and n=2 *E. coli* were *bla*CTX-M-1 and *bla*OXA-1 genes positive, respectively. Four out of six 3GCs-resistant *Citrobacter* spp. harbored a *bla*CMY-type gene. The *qnr*S gene was present in n=2 *E. coli*, n=2 *K. pneumoniae*, n=1 *E. cloacae* and n=1 *C. braakii*. Four out of 6 and 1/6 *E. coli* among the 6/14 showing a R/I phenotype to fluoroquinolones, resulted qnrS and *aac(6')-Ib-cr* gene positive, respectively. **Discussion and Conclusions:** The results highlight the presence of *bla*KPC-type, *bla*CTX-M-1, *aac(6')-Ib-cr*, and *qnr*S determinants in MDR *Enterobacterales* from surface and groundwater in Lombardy. To avoid the dissemination of such antibiotic-resistant strains and/or genes, remediation activities are urgently needed.

35 - Antimicrobial susceptibility testing of Stenotrophomonas maltophilia clinical isolates

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Introduction. Therapeutic options for treating infections caused by *S. maltophilia* are very limited due to the antibiotic resistance of this microorganism. It is naturally resistant to beta-lactams, including carbapenems. Currently, the most widely used antibiotic therapy involves the use of trimethoprim / cotrimoxazole, fluoroquinolines, tetracyclines. Anyway EUCAST breakpoint are available only for trimethoprim/cotrimoxazole

Although many show that *S. maltophilia* remains highly susceptible to trimethoprim /sulfamethoxazole, some others show an increased rate of resistance to this first-choice antibiotic.

The aim of the study is to evaluate the MICs data of 375 clinical strains of *S. maltophilia* isolated from different specimens during the period 2013-2021.

Material and methods. 375 clinical strains of *S. Maltophilia* isolated from the microbiology laboratory of the University hospital of Verona were included in the study and no multiple isolates were considere. Antimicrobial susceptibility testing was performed by Etest for trimethoprim / sulfamethoxazole, levofloxacin, ceftazidime.

Results. 96.5% (362 out of 375 isolates) *S. maltophilia* strains had MICs in the I category following the new definition of EUCAST breakpoint for Trimethoprim/sulfamethoxazole. MIC_{50} was 0.064 µg/ml and MIC_{90} was 0.125 µg/ml.

Ceftazidime MIC_{50} was 2 µg/ml, while the MIC_{90} ≥256 µg/ml. The modal MIC corresponds to 1 µg/ml. Levofloxacin MIC distribution showed MIC_{50} 0.5 µg/ml and MIC_{90} 2 µg/ml. The modal MIC stands at 0.5 µg/ml.

Conclusions. MICs data on 375 *S. maltophilia* isolates confirm the high susceptibility rate of trimethoprim/sulfamethoxazole, even some resistant strains were reported.

Based on MIC_{50} and MIC_{90} data, levofloacin showed a good activity and could be considered another therapy of choice.

23 - Characterization of antibiotic resistance mechanisms and virulence factors in Citrobacter species

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Introduction. *Citrobacter* spp. are associated with a therapeutic challenge due to the various intrinsic and acquired resistance mechanisms especially the extended spectrum cephalosporins, due to the overexpression β-lactamases. Members of the genus *Citrobacter* are emerging as important nosocomial pathogensA local or systemic breach in the host defenses can allow *Citrobacter* spp. to cause arange of infections including neonatal sepsis, brain abscess, meningitis, bloodstream and pulmonary infections. This study focused on the characterization of antibiotic resistance mechanisms and virulence factors, namely biofilm production, 32 KDa OMP and use of myo-inosytol as carbon source, in 31 strains of *Citrobacter* spp. isolated at Microbiology and Virology Service in Verona (AOUI) between 2019 and 2021 and collected from hospitalized patients of different hospital wards.

Material and methods. Strains were identified using MALDI-TOF Mass Spectrometry.

Antimicrobial susceptibility testing for cefoxitin, cefotaxime, ceftazidime-avibactam, cefepime, imipenem, meropenem, amikacin, ciprofloxacin, was performed by broth microdiluition. ESBL and Amp-C β-lactamase production were checked phenotypically by ESBL NDP test and MIC in presence of cloxacillin. bla_{CMY} gene was investigated by PCR and amplicons were sequenced.

Evaluation of Biofilm formation on plastic surface was evaluated by cristal-violet assay.

Strains investigated by protein extraction and SDS-PAGE to determine the presence of the 32-kDa outer-membrane protein (OMP) and for their ability to use myo-inositol as only carbon source by following growth curve in M9 medium added with this carbohydrate.

Results. 70,97% (22 out of 31 isolates) were found to be resistant to cefotaxime, No strain was found to be resistant to cefepime, imipenem, meropenem and amikacin.

The 22 strains resistant to cefotaxime were tested for ESBLs by ESBL NDP Test and none of them showed a phenotypic presence of ESBLs. Among these 20 strains having a positive/uninterpretable AmpC phenotypic test result, 90% (18 out of 20 isolates) were found to be positive for bla_{CMY} showing after sequencing different variant, some of them (11 out 18 sequences) show amino acid changes that are not yet reported.

The ability to form biofilm was evaluated in all the 31 strains; all of them were found to be strong biofilm producer. None of the *C. koseri* strains showed to be endowed with the 32 kDa OMP that was supposed to be involved in brain invasion, while all *C. koseri* and some *C. freundii* were able to use myo-inosytol as only carbon source

Conclusions. The main mechanism of resistance to cephalosporins in *Citrobacter* spp is related to the AmpC with a high prevalence of CMY. Different CMY variants were registered showing high variability. All strains showed to be a strong biofilm producer, a factor that may play a role in the treatment failure of the infections due to *Citrobacter* spp.

The ability of *C. koseri* and *C. freundii* strains to use myo.inosytol, sugar available at the brain level, seems to confirm to be a virulence factors that drive to these strains be involved in brain infections.

3 - Inhibition of efflux pumps restores chlorhexidine susceptibility in Acinetobacter baumannii ATCC 19606

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1. Introduction

The management of infections caused by *A. baumannii* is hindered by its intrinsic resistance to a wide variety of disinfectants. The aim of the study was to analyze the role of different *A. baumannii* efflux pumps (EPs) in chlorhexidine (CHD) resistance/tolerance mechanism.

2. Materials and Methods

MIC and MBC values of CHD were determined by a broth microdilution method. EP knockout mutants were generated by allelic replacement using pMo130-Tel^R (*sacB*+, *xylE*+) suicide vector. Biofilm formation was examined using a crystal violet staining assay.

3. Results

The *A. baumannii* ATCC 19606 strain showed a CHD MIC/MBC value of 32 mg/L and was able to grow and retain viability in the presence of subMIC concentrations of CHD. CHD subMIC concentrations increased the expression of *adeB* and *adeJ* (RND superfamily), *aceI* (PACE family) and *amvA* (MFS superfamily) EP genes. In the □*adeB* mutant and in □*adeJ*□*amvA*, or □*aceI*□*amvA* double mutants, CHD MIC/MBC decreased by 4-fold and 1-fold, respectively; triple deletion mutants showed an additive effect. Growth curves were not affected by either single or multiple EPs gene deletion; instead, the biofilm growth decreased by 30-50% in all mutants. Moreover, the carbonyl cyanide m-chlorophenylhydrazine protonophore EP inhibitor reduced dose-dependently CHD MIC in *A. baumannii* ATCC 19606 and in □*adeJ*, □*aceI* or □*amvA* mutants, but not in □*adeB* mutant. Furthermore, the MBX3756 inhibitor of RND EPs decreased CHD MIC by 2-fold in *A. baumannii* ATCC 19606.

4. Discussion and Conclusions

These results suggested that resistance to CHD in *A. baumannii* is mediated through activation of EPs, AdeB EP playing a major role. In particular, inhibition of AdeB EP and transmembrane electrochemical gradient restores CHD susceptibility in *A. baumannii*.

64 - Evaluation of Chitosan/Curcumin loaded Nanobubbles with and without Photoactivated light for Food Preservation

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Introduction

The global demand for fruits and vegetables is projected to be about US$ 2.0 trillion in 2050. Major challenges in the supply chain of fruits and vegetables are to extend storage time and to prevent the contamination by pathogenic microorganisms. Microbial contamination can occur during production, harvest, processing, storage, and distribution due to exposure of fruits and vegetables to the environment.

Fresh fruits and vegetables, for example, get spoiled within a few days due to high perishability, if not treated properly, and resulting in huge industrial losses (almost 45% loss in grown produce globally). The most efficient approach to reduce the risks of disease outbreaks and postharvest spoilage is to prevent contamination from primary sources by appropriate sanitation and decontamination methods. Chitosan-shelled Nanobubbles (NBs) based on a natural biocompatible and biodegradable polymers (i.e. curcumin), are known to exert antimicrobial effect as curcumin & chitosan having the property of antibacterial and antifungal activity. So, the present work aimed at investigating NBs antimicrobial properties against food bacteria i.e. *Escherichia coli, Staphylococcus aureus* or *Enterococcus faecalis*.

Materials and Methods

NBs was loaded with curcumin either within their core or by conjugation to the shell to enhanced antimicrobial nature against food born bacteria. The effectiveness of Curcumin/Chitosan NBs against different strains of bacteria was further enhanced by irradiating the internalized NB's with photodynamic (blue) light (optimal wavelength of 425nm, which is the proper one emitted by curcumin). Depending on the formulations (NBs) antibacterial properties were measured through microbiological and biochemical assays such as to find out minimum inhibitory concentration (MIC) and minimum bactericidal concentration.

Results

We ascertained that curcumin (alone) with blue light have a robust effect against the mentioned bacterial strains i.e. we find out MIC at a very low concentration of curcumin 0.125mg/ml, < 0.06mg/ml and < 0.015mg/ml against *E. coli*, *S. aureus* and *E. faecalis* respectively. Curcumin/Chitosan NBs have shown interesting effectiveness.

Discussion and Conclusions

Keeping under control bacterial proliferation may prolong the time in which food quality is acceptable for marketing therefore enhancing its economic value and preserving public health. Thus, it is interesting to speculate on a potential role of Curcumin NBs with photodynamic light, as to enhance antimicrobial activity and to preserve food. Our preliminary data show that Curcumin alone and NB could be useful for reducing the levels of bacterial contamination on the surface of foods, and thus be promising for applications in the field of microbiological food safety.

8 - On the main equine endometritis zoonotic pathogen: Streptococcus equi subsp. zooepidemicus.

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Introduction *Streptococcus equi* subsp. *zooepidemicus* (*Streptococcus zooepidemicus*) is a beta-hemolytic *streptococcus* belonging to the Lancefiel group C; it is a rare human pathogen but mostly in horses, it is frequently associated with endometritis. **Materials and Methods** In the year 2018, 196 uterine swabs were collected from mares suffering with bacterial endometritis and processed at the Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production, University of Naples "Federico II" (Italy). Samples were plated on different types of solid culture agar media and incubated aerobically at 37°C for 24-48 h. Moreover, the same swabs were also 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 the same agar plates. Once bacterial growth was detected, the isolates suspected to be *Streptococcus zooepidemicus* were identified by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The antibiotic resistance profiles were evaluated against 18 antibiotics belonging to 8 different classes by disk diffusion method on Mueller Hinton agar plates, according to the Clinical and Laboratory Standards Institute guidelines. **Results** The prevalence of positivity for *Streptococcus zooepidemicus* was 11.7%. Only 4 (17.4%) samples gave bacterial growth on solid media without requiring enrichment, while the other isolates (19 strains, 82.6%) were obtained only after the broth enrichment step. These strains showed high percentages of resistance to amikacin (95.6%) and other tested aminoglycosides, ampicillin (73.9%), tetracycline (69.6%). The determination of antibiotic susceptibility profiles revealed that only third generation cephalosporins, such as ceftiofur or ceftriaxone, were highly effective with 82.6% and 78.3% of the isolate inhibition, respectively. Indeed, a high prevalence of multidrug-resistant strains (82.6%) was recorded. **Discussion and Conclusions** Our results indicate that the rate of positivity for the detection of *Streptococcus zooepidemicus* in equine uterine swabs is significantly increased with the additional phase of broth-enrichment culture in comparison with direct-plating of samples. The increasing spread of multidrug-resistant *Streptococcus zooepidemicus* strains has become a relevant veterinary issue, highlighting the need of a continuous surveillance of this pathogen, in order to allow a rapid and effective antimicrobial treatment and, consequently, increase the pregnancy rate in mares. In addition, *Streptococcus zooepidemicus* has a zoonotic importance, with horses acting as reservoirs for humans. Thus, further studies on its zoonotic transmission are needed.

55 - Novel nanoconstruct based on a Choline-Calix[4]arene derivate: preliminary investigation of the mechanism of action and efficacy against antibiotic-resistant Gram negative bacteria

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Introduction. Nanotechnology is an emerging approach to generate novel antimicrobials alternative or complementary to traditional antibiotics. Due to the nanosize that increases the surface to volume ratio, a nanoconstruct may establish direct contact with the bacterial surface without the need to penetrate the cells. Previously, we reported the effects of a calix[4]arene amphiphile (Chol-Calix) bearing hydrophilic choline moieties and long hydrophobic aliphatic chains tethered to the calix[4]arene macrocyclic skeleton, on the growth, motility and biofilm of *Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 9027. Here, we performed a preliminary investigation of the mechanism of action of Chol-Calix and extended the research to antibiotic-resistant strains.
Materials and Methods. The effect of Chol-Calix on membrane permeability of *E. coli* ATCC 10536 and *P. aeruginosa* ATCC 9027 was investigated by crystal violet assay and release of 260 nm absorbing materials. The optical microscopic observation of the cells treated with Chol-Calix was performed. The susceptibility to Chol-Calix of *E. coli* NCTC 50192, carbapenemase producing *P. aeruginosa* DSM 102273 and ofloxacin resistant *P. aeruginosa* 1 ocular isolate as well as the effect on biofilm biomass of *P. aeruginosa* 1 ocular isolate were explored.
Results. The uptake of the crystal violet by *E. coli* and *P. aeruginosa* enhanced from 56% and 53% to 76.4% and 69.7% respectively, after treatment with EDTA, a known permeabilizer of the bacterial outer membrane (positive control). Analogously to EDTA, Chol-Calix enhanced the uptake of crystal violet in both *E. coli* and *P. aeruginosa* in dose-dependent manner (78.8% and 71.6% respectively at MIC concentration). No release of intracellular material was observed. The optical microscopic images showed bacteria aggregation (agglutination phenomena). Chol-Calix exhibited antibacterial activity toward the antibiotic-resistant strains with MIC and MBC values equal to 9.4 -37.6 mg/L and affected biofilm of resistant *P. aeruginosa* 1.
Discussion and Conclusions. The multiple ammonium and hydroxyl groups exposed on the surface of Chol-Calix can establish multiple interactions with the anionic sites of the membrane lipopolysaccharides and the aliphatic chains can intercalate into the membrane lipidic layer, with consequent enhancement of the permeability of the bacterial outer membrane. No cell lysis was observed and the interaction of the nanoconstruct with the surface of the bacterial cells resulted in agglutination phenomena. The antibacterial and antibiofilm properties also against antibiotic resistant strains make Chol-Calix an appealing candidate in combating resistance phenomena that are a serious threat for living beings.

162 - Whole-genome sequencing of Pseudomonas protegens Pf7: a focus on the antibiotic resistance of a potential biofertilizer

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1.Introduction. *Pseudomonas* spp. is one of the most represented genera belonging to Gamma-Proteobacteria. Some *Pseudomonas* species are plant or human pathogens, while others are known as plant growth-promoting bacteria (PGPB). In the last twenty years, PGPB have received much attention for their biological properties; they can behave as biological control agents, phytostimulators and then used in biotechnological applications in agriculture. *Pseudomonas protegens* formerly part of the *Pseudomonas fluorescens* group, is a species including free-living bacteria whose properties range from colonization of the root to competition and antibiosis.

2. Materials and Methods. Two samples of extracted DNA have been sequenced using Illumina MiSeq V3 sequencer with paired-end 300 bp reads design by an external Next Generation Sequencing facility (LGC Genomics Biosearch Technology). Proteomic analysis was performed by means of Prodigal v.2.6.3 on each reconstructed genome. Complete proteins were annotated using online web application eggNOG-mapper_v2 with default parameters.

3. Results. The genome of *P. protegens* Pf7 is composed by a single circular chromosome of 7,033,803 bp and an average G+C content of 63.2%. The genome contains 6.191 predicted protein-coding sequences (CDSs), with an average length of 997 bp. Pf7 genome includes genes for the synthesis of flagella and of cell wall, membrane biogenesis and metabolic pathway. Moreover, the genome of Pf7 contain genes involved in some physiological traits and in secondary metabolites and biocontrol activities. Genes involved in antibiotic resistance such as ampicillin and bacitracin were also detected.

4. Discussion and Conclusions. The whole genome of diverse strains of *P. protregens*, for example Pf-5, CHAO have been sequenced by next-generation and Sanger sequencing techniques. The *P. protegens* Pf7 whole genome provides relevant information for the commensal lifestyle and biocontrol capabilities and an opportunity to study its potential as plant growth-promoting bacteria.

33 - Microbiological epidemiology at the time Covid19: how much did SARS Cov2 contribute?

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1. Introduction

The new coronavirus pandemic (COVID-19) caused by the SARS-Cov-2 virus has placed unprecedented pressure on health systems. An emerging concern is the potential impact of the new coronavirus on antimicrobial resistance (AMR). In hospitals, the difficulty in clinically differentiating COVID-19 and its progression into bacterial and fungal co-infections is a significant challenge in the management of antimicrobial therapy. Clinical uncertainty is likely to drive unnecessary antimicrobial prescription in COVID-19 patients during hospitalization, potentially increasing the selection of drug-resistant infections.

2. Materials and Methods

A retrospective data analysis carried out on laboratory computer management comparing the data relative to the year 2020, of pandemic interest, with those relative to 2019, in the pre-pandemic era. Microbiological samples of patients admitted to the different departments were treated according to established procedures of microbiological practice, the relative isolates identified mainly with Vitekms/MALDI-TOF (Biomerieux), and antimicrobial sensitivity tests with Vitek2 (Biomerieux) and/or Phoenix (BD). MultidrugResistant (MDR) strains of *P. aeruginosa, A. baumannii, K.pneumoniae* and/or carbapenem resistant were tested in microdilution (Sentititre, Thermo Fisher).

3. Results

Although the workload and performance volume in the year 2020 has increased significantly due to the pandemic, the routine microbiological performance (net of the molecular test SARS Cov2), decrease of about 10%. Despite this, the bacterial isolates rates were identical to the year before, due to an increase of single patient microbiological testing from 13.8% in 2019 to 18.3% in 2020. The distribution of microbiological isolates shows a Gram negative strains decrease, equal frequency for Gram positive but an increase for fungi (from 5.5% to 7.7%). In pandemic year, compared to 2019, a significant increase of *A.baumannii* MDR, and PanDrudresistant colistin strains (PDR/COR) rising from 20% to 33%. There was a significant increase of *P.aeruginosa* isolated, however, a slight decrease of MDR strains from 35.6% to 31.6%. The *K.pneumoniae* rates, also for carbapenemase-producing strains (KPC) had a significate decrease from 44.5% to 15%. About Gram positives, *S.aureus* there was an unchanged frequency but not for methicillin-resistance susceptibility pattern (MRSA). *Enterococci*, on the other hand, record a significant increase in isolation during the pandemic.

4. Discussion and Conclusion

The epidemiological analysis of our hospital situation, in general, shows a not particularly dramatic picture in terms of the frequencies of isolated microorganisms and their resistance. Although there are some peculiar frameworks such as the increase of *A. baumannii* especially COR, mycotic infections and *E.faecalis/faecium*, *P.aeruginosa* isolates. *Enterobacteriaceae* and MRSA strains founded in deep respiratory samples, not statistically increased compared to 2019. The pandemic year

underline the importance of fast and accurate microbiological diagnosis. In this scenario is necessary, especially in the years to come, the epidemiological studies in order to understand, in the long term, whether and how the SARS Cov2 pandemic could contribute to the incidence of multi-resistant microorganisms worldwide.

158 - COVID-era antibiotic resistance data in ASL3 Sistema Sanitario Regione Liguria

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Introduction The explosive emergency of the SARS-CoV-2 virus in early 2020 overshadowed many other health issues, including the most urgent in infectious disease, the antibiotic resistance (AMR). During the pandemic, an increase in multidrug-resistant germs (MDR) has been observed in many situations, especially in the intensive care units. The problems encountered at the beginning of the pandemic such as the appearance of a new disease with severe clinical pictures, findings similar to those of a bacterial infection, lack of diagnostic tests, explain the complete disruption of the principles of antimicrobial stewardship. This is associated with empirical antibiotic treatment in this type of patient based on the experience of a potential bacterial overlap in influenza virus infection.

Materials and Methods. The Microbiology laboratory of ASL3 examines biological samples from 5 hospitals. The aim of this study is to evaluate the trend of pre and post COVID-19 AMR, focusing on hospital departments. In particular, we analyzed surveillance and isolations of gram negative bacteria. The data was extracted using the Mercury Noemalife epidemiological software.

Results Table 1. Comparison of the percentages of positivity of the surveillance 2019 and 2020.

	2019		**2020**	
	TOT	**POS**	**TOT**	**POS**
CRE (rectal swab)	4657	153 (3.29%)	3353	128 (3.82%)
ESBL (rectal swab)	298	69 (23.15%)	308	64 (20.78%)
MRSA (nasal swab)	297	46 (4.89%)	309	32 (3.76%)
VRE nasal swab)	940	0	851	2 (0.65%)

Table 2. Antibiotic resistance profiles of Gram negative bacteria isolated in 2019 and 2020 (% resistance)

	E. coli		***P. mirabilis***		***K.pneumoniae***		***P. aeruginosa***		***A. baumannii***	
	2019	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
Amikacina	0,1	0,3	0,2	1,2	10,9	15,1	10,9	11	26,1	70,6
Amoxicillina/Clavulanico	56,5	28,4	32,5	25,9	52,7	14,6				
ESBL	35,8	35,8			36,7	23,9				
Cefepima	22,6	17,7	3,3	4,1	29	39,9	38,7	31,9		
Cefotaxima	33,6	27,2	25	20,3	52,1	44,7				
Ceftazidima	29,1	11,3	25,7	5,6	49,4	21,9	24,1	22,5		
Ciprofloxacina	48,2	40,7	53,8	55	46,1	45	34,4	35,2	39,1	73,5
Colistina	41,8	8,9	99,8	100	22,2	23,5	9,6	6,7	0	0
Cotrimoxazolo	37,8	32,4	60	57	36,7	39,6			17,4	61,8

Ertapenem	1,8	1,4	3,2	0	18,5	26,2				
Fosfomicina	2,5	4,9	22,3	31	34,7	29,9				
Gentamicina	16,5	13,8	35,7	27,5	27	18,1	17,1	16,7	30,4	61,8
Meropenem	0,3	0,2	0,5	0	16,4	21	23	24,5	26,1	58,8
Piperacillina/Tazobactam	15,2	11,3	1,5	1,6	36,1	38	37	41,4		

Discussion and Conclusions. From our data there is not an increase in AMR in 2020 compared to 2019, neither with regard to the isolation of sentinel pathogens in surveillance, nor with regard to gram negative bacteria isolated from biological samples. The only exception was an outbreak of *A.baumannii* in intensive care unit with 12 isolations of which 9 XDR.

It will be necessary to continue this monitoring and to support the principles of antimicrobial stewardship, coordinating interventions and promoting the appropriate use of antibiotics even in these difficult times.

153 - Screening a repurposing library for the discovery of drugs with antimicrobial and antibiofilm effect against Pseudomonas aeruginosa from cystic fibrosis patients

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Introduction. *Pseudomonas aeruginosa* is the main pathogen responsible of chronic airways infections in cystic fibrosis (CF) patients. The occurrence of acquired and biofilm-related drug-resistance accentuates the need to identify novel antibiotics. We wanted to identify potential novel anti-*P. aeruginosa* therapeutics by screening a drug repurposing library for antibacterial and antibiofilm activities under experimental conditions relevant to CF.
Materials and Methods. The Drug Repurposing Compound Library (HY-L035; MedChemExpress, Sollentuna, Sweden), consisting of 3386 approved and clinical drugs, was screened at 0.1 mM to identify "hit" compounds against *P. aeruginosa* RP73, a model strain to investigate long-term persistence in CF lung infections.
Both antibacterial and antibiofilm effects were evaluated in 96-well polystyrene plates 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), whereas a crystal violet staining assay was carried out for antibiofilm activity evaluation. A potential "antibacterial hit" (a-BACT) was identified for OD_{620} or OD_{492} reduction of $\geq$ 90%, whereas a potential "antibiofilm hit" (a-BIOF) had to inhibit biofilm formation $\geq$ 75% and showed $\leq$ 10% effect on planktonic cells.
Results. Our initial screening identified a total of 23 a-BACT (23 out of 3386, 0.6%) and 15 a-BIOF (15 out of 3386, 0.4%) drugs that have been developed for therapeutic indications other than antibacterial, suggesting that they have the potential to be repurposed as antibacterial agents toward *P. aeruginosa*. Hits were classified according to their therapeutic indications and belonged to the following categories: cancer (a-BACT: 12; a-BIOF: 6); anti-infection - i.e., antiparasitic, antifungal or antiviral (a-BACT: 6); neurologic disease (a-BACT: 2; a-BIOF: 1); metabolic disease (a-BACT: 1; a-BIOF: 1); cardiovascular disease (a-BACT: 1; a-BIOF: 3); inflammation (a-BIOF: 1); endocrinologic disease (a-BIOF: 1); and miscellaneous (a-BACT: 1; a-BIOF: 2). The clinical information of the hits was as follows: launched (a-BACT: 15; a-BIOF: 12), phase 2 (a-BACT: 3; a-BIOF: 1), phase 3 (a-BACT: 5; a-BIOF: 1), and phase 4 (a-BIOF: 1).
Discussion and Conclusions. Repurposing of approved drugs is a viable alternative to *de novo* drug discovery and development. Our results defined some drugs that, acting on bacterial viability or virulence inhibition, 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.

106 - Microorganisms isolated from wounds and study of antimicrobial resistance

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Introduction. Pathogens can be responsible for skin and wound infections and their capability of developing biofilm can prolong the healing time and promote antimicrobial resistance events. The antimicrobial resistance is a problem of global interest in the treatment of clinical infections, therefore, the goal of this retrospective study was the identification of the microorganisms responsible of skin infections, as well as the determination of the drug susceptibility pattern associated.
Materials and Methods. The present retrospective study has been performed over three years (from 2017 to 2019) on skin wound samples collected by the microbiology laboratory of San Pio Hospital, Vasto (Chieti, Italy). The study included 239 patients with an average age of 69 years that presented just a skin wound. The microorganisms responsible for the infections were isolated by culture methods, identified through biochemical tests and analysed for their susceptibility patterns to antimicrobial drugs through the Walk Away automated system.
Results. The presence of only one species isolated from each sample was the most frequent condition (75.3% of infected wounds). On the contrary, the co-infection associated with different microorganisms was detected in 24.7% of samples. The data showed that the most common bacterial species detected were Gram-negative (58.6%) and, among these, *Pseudomonas aeruginosa* (39.3%), *Escherichia coli* (20.0%), *Proteus mirabilis* (13.6%) and *Acinetobacter baumannii/haemolyticus* (11.4%). Gram-positive bacteria were observed in 38.9% of samples and the predominant species was *Staphylococcus aureus* (77.4%). Regarding the antimicrobial susceptibility pattern, we detected the presence of at least one resistance in 86% of isolates. In particular, Ampicillin was the antimicrobial toward which the microorganisms showed the higher percentage of resistance corresponding to 54.4%, followed by Penicillin (27.2%), Trimethoprim/Sulfamethoxazole (24.7%), Piperacillin (24.3%) and Gentamicin (23.8%).
Discussion and Conclusions. Limiting the spread of antibiotic resistance is a public health goal to be pursued and requires the control of multi-resistant bacteria and the availability of revised therapeutic regimens. Wound's infections are underestimated problems that result into a chronic disease. This study shows that in most cases, infection develops at a wound site and this infection is often associated with at least one microorganism resistant to an antimicrobial. The presence of polymicrobial infections and biofilm formation make the microorganisms eradication more difficult. Therefore, accurate microbiological analyses, followed by a proper therapeutic treatment, lead both to the resolution of the infection and to the wound healing.

142 - Farnesane-Type Sesquiterpenoids with Antibiotic Activity from Chiliadenus lopadusanus.

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INTRODUCTION - Antimicrobial resistance is a phenomenon that seriously endangers the control of diseases around the world. The clinical efficacy of many classes of antibiotics is at this point compromised with a consequent increase in mortality associated with infections. Furthermore biofilm-related infections represent a major global problem in the hospital setting due to their intrinsic recalcitrance toward antibiotics and to difficulties in treatment. The development of new antibiotics proceeds very slowly, making it necessary to search for new potential antimicrobial scaffolds from different sources. Plants are a rich reservoir of compounds with several biological activities, including antimicrobial properties. A screening on endemic plants collected in different regions of the Mediterranean basin was aimed to find new antibiotics. Among the corresponding organic extracts, that of *Chiliadenus lopadusanus Brullo*, an Asteraceae plant species endemic to Lampedusa island, showed a growth inhibiting activity against some human pathogenic bacteria. Therefore, the extraction, the purification, and the chemical characterization of the main metabolites produced by *C. lopadusanus* was carried out and their antimicrobial activities against *Staphylococcus aureus* and *Acinetobacter baumannii* were assayed.
MATERIALS AND METHODS – The organic extract of *C. lopadusanus* whole aerial parts was fractionated employing bioguided purification procedures affording three main farnesane-type sesquiterpenoids, identified by spectroscopic methods. The antibacterial and antibiofilm activities of these sesquiterpenes were tested on reported strains through standard microdilution method and crystal violet assay respectively.
RESULTS - For the first time, our study reports the isolation of the three sesquiterpenes, namely 9-hydroxynerolidol, 9-oxonerolidol, and chiliadenol B, from *C. lopadusanus Brullo*. The compounds were tested individually against the reported strains. Both 9-hydroxynerolidol and 9-oxonerolidol showed antibacterial activity identifying the minimal inhibitory concentration and minimal bactericidal concentration values. Despite the lack of activity against planktonic cells, chiliadenol B was instead the only one able to inhibit the biofilm formation of both strains.
DISCUSSION AND CONCLUSIONS - Plant extracts can have a good activity on their own or can be sources of antimicrobial compounds effective against important multidrug-resistant and biofilm-forming pathogens. Therefore, *C. lopadusanus Brullo* could be used as a source to isolate secondary metabolites as potential new antibiotics and it would be of considerable interest to evaluate the possible use of a combination of the 3 compounds isolated from the plant for the prevention of biofilm-related infections.

144 - Nanofilm: a new drug delivery system to counteract Multi Drug Resistant Gram negative bacteria infections

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Introduction: Evolution of resistance to last-resort antibiotics, such as colistin (Col), in multidrug–resistant (MDR) Gram-negative bacteria (GNB), including *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, causes a relevant problem in the treatment of different nosocomial infections as the skin and soft tissue infections (SSTI). Nanocarriers have been proposed for antibiotic delivery as a promising strategy to counteract resistant infections increasing drug efficacy and penetration. This work aimed at developing a topical formulation of colistin-loaded albumin nanoparticles for the treatment of MDR GNB SSTI infections. **Materials and methods:** We developed a formulation of chitosan-coated human albumin nanoparticles for the Col delivery (Col/haNPs) and evaluated the antimicrobial and antibiofilm activity as well as the biocompatibility. Starting from this preparation it was developed a topical formulation based on a film forming spray (consisting of glycerol, etilacetate and the copolymer Plastoid® B) and the Col/haNPs, called "NANOFILM". The formulations were characterized and biocompatibility was performed by MTT assay on human fibroblasts and human skin and by haemolytic activity determined spethrophotometrically on red blood cells. The broth microdilution test was performed according to EUCAST guidelines to evaluate MIC on different MDR GNB (*A. baumannii*, *P. aeruginosa and K. pneumoniae*) and the effect on biofilm formation was assessed by crystal violet staining method. An ex vivo skin infection model was performed to evaluate the antibacterial effect of the topical formulation. **Results:** the Col/haNPs showed sizes lower than 200 nm, an high encapsulation efficiency and a prolonged in vitro release of Col. The safety of the nanoformulations was demonstrated by no cytotoxicity on epithelial cells and human skin and by no hemolytic activity. Both Col/haNPs and the NANOFILM can reduce the MIC values respect to free Col, in both MDR Col R and Col S strains. Moreover the Col/haNPs displayed a potent biofilm inhibition and significantly reduced the biofilm also at 1/2 MIC. By contrast Col free is able to reduce biofilm only at higher concentrations. The antimicrobial effect was also demonstrated in an ex vivo skin infection model using *A. baumannii* and the NANOFILM carrying the nanoparticles. **Discussion and Conclusions:** SSTI caused by MDR GNB are becoming increasingly prevalent and constitute a global problem because they are difficult to treat and are associated with high morbidity and mortality rates in patients with underlying immunodeficiency, as well as burn or trauma-related injuries. Our findings suggest that Col/haNPs represent a promising nanocarrier for Col topical delivery with high antimicrobial activity on MDR GNB.

42 - Antimicrobial activity of zinc-doped hydroxyapatite coatings formed on titanium Ti6Al4V surface for orthopedic implant

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Introduction

Prosthetic joint infection (PJI) is one of the most serious complications of prosthetic joint implantation leading to a longer hospitalization. S. *aureus* is the predominant cause of PJI followed by *Pseudomonas aeruginosa* and *Stafilococci* coagulase negative. Several studies focused on the development of effective antibacterial surfaces that prevent bacterial adhesion, colonisation and proliferation into the surrounding tissues and it has been widely demonstrated that zinc ions (Zn^{2+}) exhibit antimicrobial activity against various bacterial and fungal strains. The aim of this study was to evaluate the *in vitro* activity of Zn^{2+} generated from the partial dissolution of Zn particles on surface of titanium discs, against *S. aureus* ATCC 29213.

Materials and Methods

Hydroxyapatite (HA), and HA/Zn^{2+} doped discs (diameter:1,7cm) were used. The protocol used is still reserved. Each disk (three for each group) was inoculated with suspension of S. *aureus* ATCC 29213 (10^4 Colony Forming Units/ cm_2 - CFU/cm^2) prepared in agar slurry, following standard ASTM (American Society for Testing and Materials) E2180-07 method. After initial exposure, control samples were immediately processed in order to know exactly the starting inoculum (T0). The disks were transferred into the tubes cointaining the neutralization media and vortexed to remove the bacteria from the surface. The sospension was serially diluted and plated on TSA agar. The other samples (untreated and treated) were incubated for 6 hours at 37°C. After 18 h of incubation at 37°, CFU of all the samples were counted. The antibacterial activity was calculated as follows: %Reduction= [A-B/A] x 100, where A=average of the logarithm of the number of viable bacteria after initial bacterial exposure (T0); B=average of the logarithm of the number of viable bacteria of the disks after time contact. To confirm quantitative data, morphological analysis was performed by Scanning Electron Microscope (SEM).

Results

The HA-Zn^{2+} disks showed a 2.9 $\log_{10}$±1.9 (99,8%) CFU decrease ($p<0.05$), after 6 hours of incubation. The SEM analysis of the surface of HA disk, revealed bacteria with the typical spherical shape, uniformly distributed; the Zn^{2+} doped disk showed small colonies of about 2-10 bacteria.

Discussion and conclusion

Here we demonstrated that HA-Zn^{2+} coating has a very good antimicrobial activity against *S. aureus*, also confirmed by SEM. The novelty of this study consists of a new process that allows to incorporate the zinc during the standard process of orthopedic implants HA coating. Further studies will focus on the investigation of the activity against other microorganisms involved in prosthetic infection disease, before and after biofilm formation.

53 - Effect of the novel siderophore cephalosporin Cefiderocol on iron transport system in A. baumannii strains under iron starvation

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Introduction: Cefiderocol (CFD) is a novel siderophore cephalosporin, approved for the treatment of infections due to aerobic Gram-negative microorganisms, containing a cephalosporin core with a catechol siderophore moiety on the C-3 side chain that chelates ferric iron (Fe^{3+}). According to EUCAST guidelines, CFD *in vitro* activity must be tested in Iron-depleted Cation Adjusted Mueller Hinton broth (ID-CAMHB) to allow the CFD entering cells. We planned to reveal, by RT-qPCR and RNA-seq, the different expression of genes involved in iron transport as a response to iron deficiency and CFD activity in *A. baumannii* (*Ab*) strains with diverse resistance profile to CFD. Here we present a preliminary part of the study.

Material and Methods: 2 CFD sensitive (1S, 2S), 3 resistant (1R, 2R, 3R) and 1 hetero-resistant (HR) *Ab* strains were selected to be analyzed for their molecular response to iron depletion and CFD action. All the strains were grown to their exponential growth phase in CAMHB (Fe^{3+} 0,084 ng/µL) and ID-CAMHB (Fe^{3+} 0,005 ng/µL) in the presence and in the absence of CFD. RNA was analyzed by RT-qPCR with specific primers targeting *exb*D, *bau*A, *iuc*A/C and *ton*B genes as they are part of the siderophore biosynthesis and transport system. The Glutamine-fructose-6-phosphate aminotransferase (*glm*S) gene expression was also evaluated as a measure of bacterial stress. Data were analyzed using one-way ANOVA, followed by Bonferroni's multiple comparison test, on GraphPad Prism.

Results: Our RT-qPCR preliminary data showed an up-regulation of *iuc*A/C and *ton*B genes in 1S and 1R strains when CFD was added to normal CAMHB. An up-regulation of all the genes, but *ton*B, was detected in 1S, 1R and 2R strains when grown in ID-CAMHB. Adding CFD to ID-CAMHB led to the downregulation of *exb*D in 1S and 2R strains, *bau*A in 1S, 2S, 1R and 2R strains, *iuc*A/C in 1R and 2R strains and *ton*B in the HR strain. *glm*S expression did not vary in all conditions.

Discussion and Conclusions: These preparatory results highlight an increased activity of iron channels and transport system in an iron deficient condition, and a detrimental effect of CFD on the same system, more evident in the CFD resistant *Ab* strains, when bacteria have limited access to iron due to its lack in the growth medium. Interestingly, the HR strain seems to be the only one with a *ton*B down-regulation in the presence of CFD, maybe due to its particular membrane lipidic localization. The unvaried expression of *glm*S suggests that the other gene different expressions are not due to bacterial stress. Further experiments are planned, both by RT-qPCR and RNA-seq, in ID-MHCAB supplemented with a high ferric iron concentration to verify if this will restore the iron channel functionality and how this can affect the CFD effects.

92 - Heteroaryl-ethylenes as new effective agents for high priority bacterial clinical isolates

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Introduction The World Health Organization (WHO) has identified antimicrobial resistance as a matter of concern for the public authorities in both developed and emerging countries and developed a global priority pathogens list of antibiotic-resistant bacteria. Amongst the Gram-positive species, *Staphylococcus aureus* (methicillin-resistant - MRSA, vancomycin intermediate and resistant - hVISA and VISA) and *Enterococcus* spp. (vancomycin-resistant or VRE) were classified at high priority. We aim to define new antimicrobial candidates against difficult-to-treat Gram-positive pathogens starting from an in-house database of heteroaryl-ethylenes. The ability of the PBn compounds to inhibit the proliferation of bacterial cultures was evaluated by carrying out Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) on a selected sample of *S. aureus* and *Enterococcus* spp. clinical isolates, selected for different antibiotic profiles.
Materials and Methods. The tested molecules were synthetized via Knoevenagel Condensation using the proper heteroaromatic aldehyde. PB compounds were tested on control strains including *S. aureus* ATCC12598 and *E. faecalis* ATCC29212, and on clinical strains including 18 *Staphylococcus aureus* and 16 *Enterococcus spp.* MIC and MBC of the PB molecules were performed according to the standard method, with some modifications. All the compounds were at the concentration of 8,000 mg/L in 100% Dimethylsulfoxide.
Results Later the *in-silico* evaluation of the antibacterial effects of the compounds through the Volsurf+ software, we measured the microbiological activity expressed as MIC on ATCC and the Multi Drug Resistant (MDR) clinical strains. Compounds PB4, PB5, PB7 and PB8 showed the best values in terms of MIC (0.125-16mg/L) and were also evaluated for MBC confirming a bacteriostatic activity. The PB4 was the most effective molecule, which gives lower MIC values on all strains tested in particular of *Enterococcus spp*.
Discussion and conclusions. Biological assays have shown for these derivatives an excellent bacteriostatic activity. These remarkable results have indicated a greater efficacy of the compounds, mostly PB4, so it was promising for the present research and worth of further investigation. These novel compounds may be both safe and effective in the treatment of many Gram-positive bacterial infections, especially those caused by MRSA and VRE. The next step in the study of these molecules will certainly involve cross-tests, in which the inhibition of bacterial and cellular replication will be evaluated at the same time, but we will also try to understand what are the mechanisms by which these compounds exert their antibacterial activity.

74 - Genotypic characterization of clinical isolates of Listeria monocytogenes

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Introduction: The gram-positive bacterium *Listeria monocytogenes* is an ubiquitous, intracellular pathogen which has been implicated within the past decade as the causative organism in several outbreaks of foodborne disease. Listeriosis, with a mortality rate of about 24%, is found mainly among pregnant women, their fetuses, and immunocompromised persons, with symptoms of abortion, neonatal death, septicemia, and meningitis.
Materials and methods:The purpose of this study was to characterize from a genotypic and phenotypic point of view 26 clinical strains of L. monocytogenes isolated from patients hospitalized at the main Palermo hospitals and to evaluate their susceptibility profile to antibiotics. In particular, a molecular characterization of the isolates was carried out with the use of multiplex PCR to evaluate the presence/absence of the main genes included in the four Listeria pathogenicity islands (LIPI) and the serotype. All strains were analyzed through Multilocus sequence typing (MLST), Multi-virulence-locus sequencetyping (MvLST) and the susceptibility profiles to antibiotics were evaluated by automated methods.
Results: The results obtained from serotyping by multiplex PCR of the isolates confirm that the prevailing serotype is 4b (0.96%), while only one belongs to the serotype 1/2a (0.04 %), which in the literature is indicated as the one mainly isolated from food sources. The results obtained by molecular intraspecies typing show that the prevailing SequenceType among the strains with serotype 4b is ST2 (0.92%), followed by the ST6 shown from the only isolate with serotype 4b and which has LIPI-3 responsible for the synthesis of the virulence factor listeriolysin. The only isolate with serotype 1/2a shows ST155. The results of the Multi-virulence-locus sequence typing (MvLST) revealed that 96% of the test isolates belong to VT21 while the strain with ST6 and with ST155 show VT19 and VT45 respectively. Furthermore, the presence of virulence genes contained in LIPI pathogenicity islands (Listeria monocytogenes Pathogenicity Island) 1 and 2 was observed in all clinical isolates, while LIPI-3 was present only in a clinical isolate with serotype 4b (ST6/VT19), and LIPI-4 in no clinical isolate under investigation. All the clinical isolates showed a very similar susceptibility profile to antibiotics, independent of serotype and intraspecies typing.
Discussion and Conclusion: In order to reduce the incidence of this bacterium throughout the food chain, it is necessary to strengthen the surveillance network which must include the reporting of confirmed cases of human listeriosis, the centralized collection of data on the characterization of *L. monocytogenes* and the shared best practices to control the food-borne outbreaks.

05 Microbioma

95 -Lungs from COVID-19 patients harbor a potential immunogenic microbiota

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Introduction. We recently published compositional differences in the oral microbiota from COVID-19 patients, but the observed dysbiosis warrants further investigations, especially considering the existing link among oral and lung environments. In the present study we aimed at demonstrating the putative differences among oral and lung microbiota species, and among oral and lung cytokines.
Materials and Methods. Two cohorts of hospitalized COVID-19 patients (n=16 for bronchoalveolar lavage fluid – BAL; n=26 for oral samples) and matched healthy controls (n=15) were enrolled. Microbiota composition was analyzed by 16S rRNA-V2 region targeted sequencing, using the Ion Torrent PGM platform. Levels of 27 cytokines from oral and BAL were assessed using magnetic bead-based multiplex immunoassays, via the Bio-Plex 200 instrument. Dedicated bioinformatic pipelines and multivariate statistics coupled to network analysis were employed to cross-relate all datasets.

Results. COVID-19 lung and oral microbiota differed for beta-diversity (PERMANOVA = 4.488; P=9.9*10^{-4}), while exhibiting a similar bacterial species richness. A greater prevalence (44%) and abundance (13%) of the usually rare and immunogenic intestinal species *Enterococcus hirae* was found in BAL, while its levels were 96% lower in the mouth (P=1.17*10^{-3}). Other species preponderant in BAL (*Streptococcus_mitis*, *Prevotella_oris*, *Prevotella_salivae*) are common resident within the oral habitat, while the endocarditis- and sepsis-related *Staphylococcus_haemolyticus* is from the skin. Cytokines were able to divide lung and oral environment (PERMANOVA = 51.573; P=9.9*10^{-4}). Morover, we found that IL-6 and IL-5 were distinctive for the lung of COVID-19 patients (P_{mean}=5*10^{-7}), while GMCSF was sufficient to discriminate the oral environment (P=1.8*10^{-4}). Interestingly, a significant linear correlation was found among lung and oral cytokines (Pearson r=0.99, P=5.3*10^{-13}).

Discussion and Conclusions. Our pilot study evidenced a different microbiota composition among oral and lung environment, as expected from the literature (Human Microbiome Project), and such a difference was not overwhlemed by the selective pressure exerted by the COVID-19. Some lung species belonged to the oral environment, while one intestinal species, interesting for its immunogenic potential (*E. hirae*), is sometimes isolated from BAL. We speculate that, given the almost perfect linear correlation among lung and oral cytokines, some distinctive bacterial species would be amenable of particular research to mitigate the "cytokinic storm" present in both these COVID-19 patients' districts.

161 - The ecology of the microbiota in children with obesity is associated to insulin resistance and diet composition

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Introduction. In the pediatric population, the progression of obesity-related diseases can be delayed or prevented through lifestyle changes, including the promotion of a Mediterranean-like dietary (MD) pattern.
Objective. We aimed to evaluate the gut microbiome ecology in relation to dietary and clinical parameters in the pediatric subjects with obesity recruited at baseline in a protocol on an educational training to MD.
Methods. A total of 55 subjects (6 and 18 years) with obesity, diet naïve or with failure to a previous weight loss program were recruited. We collected auxological, metabolic, nutritional parameters (KIDMED score; IDEFICS food frequency questionnaire), and stool samples. DNA was extracted directly from 0.25 g of stool using the Power SoilKit. DNA was amplified with primers for the V3 and V6 regions of 16S rDNA tagged with Multiplex Identifier sequences using Microbiota Solution B Kit optimized for Illumina Miseq sequencing. Raw FastQ sequences were analyzed using MicrobAT Software. Statistical analyses were performed using R software.
Results: All the 55 subjects showed a Bacteroides enterotype: 38% Bacteroidetes, 34% Firmicutes, 22% Unclassified Bacteria, 4% Actinobacteria, 1% Proteobacteria. At baseline, clinical and metabolic characteristics were homogeneous among children while microbial communities associated with the different subjects showed statistically significant differences according to age, Tanner stage considering sex, fasting insulin levels, fasting insulin resistance (HOMA 95° percentile), percentage of carbohydrates, and activity. In particular, considering HOMA 95°, *Bifidobacterium pseudocatenulatum*, unclassified Faecalibacterium, Bifidobacterium sp., Unclassified Sutterella, and unclassified Blautia were correlated with higher insulin resistance while *Dialister invisus* and Barnesiella sp. were associated with lower insulin resistance. Moreover, we observed *Bacteroides dorei*, *Bacteroides vulgatus*, Unclassified Ruminococcaceae, and *Faecalibaterium prausnitzii* associated with a higher carbohydrate intake. Finally, with a higher activity rate, we observed an increase in *Collinsella_aerofaciens, Parabacteroides merdae, Bacteroides merdae, Ruminococcus bromii, Bifidobacterium longum.*
Discussion: These preliminary results highlight as diet, insulin sensitivity and microbiome are strictly related also in children with obesity. We identified several bacterial groups not previously described in obesity. These findings are of importance for clustering patients and studying tailored dietary programs.

29 - Impact of Bacillus cereus on an in vitro model of the human gut microbiota

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Introduction: The use of *in vitro* models of the gut microbiota has become essential to study the dense network of interactions between microbes and analyze the impact of perturbations on microbial balance. In this study, a 3D model of the human gut microbiota was used to evaluate the effects on the intestinal bacterial communities of the human pathogen *Bacillus cereus*, commonly causative agent of food-poisoning.

Materials and Methods: Gelatin structures used as scaffolds for microbial growth were created by electrospinning and some of them covered by mucins. The fecal microbiota, prepared according to the European Guidelines for fecal microbiota transplantation, was inoculated on the gelatin scaffolds and incubated in anaerobic atmosphere for up to 72 hours. After 24h of incubation, *B. cereus* or its culture supernatant was added. At different time intervals, samples were subjected to electron scanning microscopy, live/dead imaging, and crystal violet adhesion assay to highlight microbial ability to survive and multiply during *in vitro* culture and to adhere to scaffolds. The microbial communities grown on the scaffolds were analyzed for composition and amount by 16S metagenomic sequencing and Real-Time qPCR.

Results: The gut microbiota was able to proliferate, adhere to scaffolds, and form long-lasting biofilms, preferentially in the absence of mucins. When *B. cereus* was added, a significant reduction in the adhered biomass was observed. Differences in the composition of the bacterial communities were pointed out at the *phylum* and *genus* level. Abundance of *Proteobacteria* was strongly reduced after the addition of both *B. cereus* and its culture supernatant. In the presence of mucins, the level of *Actinobacteria* was slightly increased at 48h when the pathogen was added and the amount of *Bacteroidetes* was lower following the addition of the *B. cereus* supernatant after 72h. No further differences in the main *phyla* were highlighted. More quantitative fluctuations were observed when *genera* were considered, especially for *Escherichia*, *Mitsuokella*, *Bifidobacterium*, *Roseburia*, and *Lactobacillus*. Overall, the introduction of *B. cereus* interfered with the gut microbiota ability to adhere to the gelatin scaffolds and modulated the composition of the *in vitro*-cultured community.

Discussion and Conclusions: The *in vitro* culture method used in this study appears suitable for propagating the gut microbiota and analyzing the impact of perturbations (e.g. pathogens, drugs, dietary components) on intestinal microbial communities. Our data obtained after the introduction of *B. cereus* in the model suggest that this pathogen, besides causing harm to host cells, is also able to directly impact on the human gut microbiota.

160 - Effect of food supplement on gut microbiota: preliminary results of ZIMBA Trial

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1- Introduction. Human bodies harbor a diverse community of microbes that together compose the human microbiota. The microbiome is defined as the collective genomes expressed by the microbiota. For years, scientists have been interested in gut microbiota, but one of the major difficulties in the relevant research was to study not cultivable bacteria. New technologies allowed researchers to identify and/or quantify the components of the gut microbiota by analyzing nucleic acids directly extracted from stools. Prebiotics are a group of nutrients that are degraded by gut microbiota and their relationship with human health has been an area of increasing interest in recent years. They can feed the intestinal microbiota, and their degradation products are short-chain fatty acids that are released into blood, affecting not only the gastrointestinal tracts but also other distant organs.

2- Materials and Methods. Zimba project is a double-blind placebo-controlled clinical trial that study the efficacy of the association of Zinc and Myoinositol and GOS (treated-patients) in respect to GOS (placebo-patients) in pediatric obesity. The genomic DNA was extracted using the DNeasy® PowerSoil® kit (Qiagen). The extraction took place starting from 0.25 g of stool following the manufacturer's instructions. The bacterial 16S DNA libraries were prepared using the Microbiota solution B kits (hypervariable regions V3-V6) provided by Arrow Diagnostics srl. (Genoa, Italy). The amplicon pool was processed using the MS-103-1003-MiSeq Reagent Nano Kit v2 kit, supplied by Illumina. Bioinformatic analysis workflow was according to Bona et al., 2021. Briefly, obtained raw sequences were processed with the software MicrobAT v. 1.2.0 provided by UPO-SpinOff (SmartSeq srl, Novara, Italy). Statistical analysis was performed using both MicrobiomeAnalyst and R softwares.

3- Results. The microbiota characterization of 27 enrolled patients (15 active patients and 12 placebo) – was presented here. Preliminary results showed a decrease in biodiversity in active treated patients in respect to placebo ones, probably due to Zn. Moreover, the prebiotic intake alone induced a significant increase in Firmicutes: *Dorea longicatena, Clostridium colstridioforme* and *Faecalibacterium sp*. and the decrease in Bacteroides, *Bacteroides caccae.* Finally, the increased abundance of unclassified *Bifidobacterium* in the two groups, but greater in the placebo group, was observed.

4- Discussion and Conclusions

The biodiversity reduction observed in the composition of the treated patient microbiota could be ascribed to the Zn effect. On the contrary, the prebiotic intake alone positively influenced the abundance of *Dorea longicatena*, essential for a healthy microbiota homeostasis, *Clostridium clostridioforme,* a member of normal intestinal microbiota, and *Faecalibacterium sp*. that has been considered as a bioindicator of human health, because when its population is altered (decreased), inflammatory processes are favored.

Concluding, increase the number of enrolled patients and further analyses linked to the clinical and metabolic responses will be necessary to validate the proposed mechanisms at the base of the microbiota modulation occurring in the two populations.

101 - Oral microbiome and local immune/inflammatory response in COVID-19 patients: a cross-sectional study.

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Introduction: The new human coronavirus SARS-CoV-2, responsible for the development of COVID-19 disease, has become a global pandemic emergency. Like other respiratory viruses, the primary site of the entrance is represented by the oropharynx, and the local microbiome environment may influence its capability to infect and induce the disease. The aim of the present study was therefore to characterize the oral microbiome in a cohort of COVID-19 patients with different symptom levels, to evidence the eventual association between virus-induced disease and the microbial environment of the oral cavity. Moreover, the inflammation and local immune response were also assessed in parallel.
Materials and Methods: Overall, 75 oral rinse samples were collected from 39 COVID-19 subjects and 36 controls recruited in the study. Each specimen was reassessed by digital droplet PCR to measure the load of SARS-CoV-2 at the time of withdrawal. The profile of the oral microbiome was analyzed by Whole Genome Sequencing (WGS), allowing evidencing also the non-bacterial components (mycobiome and virome) of the oral microbiome. In parallel, the local immune response (secretory IgA) and inflammatory cytokine release (IL-6, IL-17, TNFα, and GM-CSF) were assessed by specific ELISA assays.
Results: WGS results showed significant alpha-diversity decrease in the oral microbiome of COVID-19 patients compared with matched controls, associated with symptom severity, and oral dysbiosis was associated with the increased local concentration of inflammatory cytokines and decreased mucosal secretory IgA response. Bacterial genera associated with poor oral hygiene and periodontitis were increased in COVID-19 subjects (*Prevotella*, *Capnocytophaga*, *Porphyromonas*, *Abiotrophia*, *Aggregatibacter*), with *Enterococcus* and *Enterobacter spp.* exclusively detectable in COVID-19 patients. In addition, also mycetes (*Candida*, *Saccharomyces*), and viruses (EBV, HSV-1) were significantly increased, with *Aspergillus*, *Nakaseomyces*, and *Malassezia genera exclusively detectable in COVID-19 patients.*
Discussion and Conclusions: The oral microbiome may be important in defining the individual susceptibility to SARS-CoV-2 infection and the subsequent development of symptomatic COVID-19. In particular, poor oral hygiene might facilitate inflammation and a worse course of COVID-19 disease. Instead, sIgA presence associated with mild symptoms may be considered as an important marker in monitoring therapy and vaccine development.

95 - Microbiome and bacterial vaginosis: which relationship?

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Introduction: Bacterial vaginosis (BV) is a vaginal dysbiosis affecting one-third of reproductive age women, increasing the risk of acquiring sexually transmitted infections (STIs) and posing a risk for reproductive health. The vaginal microbiome and the local immune response are considered critical factors with a strong impact on women's health. This study aims to evaluate the vaginal microbiome and the local immune mediators in the context of the vaginal Community State Type (CST) classification and of the current diagnostic tool for BV. Materials and Methods: A total of 90 vaginal samples from asymptomatic women not reporting any vaginal complaints, were retrospectively evaluated for microbiome assessment and immune factor profile by the Ion Torrent PGM and the Luminex Bio-Plex technologies, respectively. Moreover, we analyzed cervicovaginal swabs from 985 symptomatic women (vaginal discharge, burning, itching) by Nugent score and qPCR for BV, aerobic or fungal vaginitis and STIs microorganisms.

Results: the CST microbiome classification together with the local immune status represented a good predictive indicator of the vaginal health, suggesting the strong association of a specific *Lactobacillus* with each CST status. To note, the colonization by *Bifidobacterium* may absolve a protective role similar to that of *Lactobacillus*, suggesting a possible new CST. Within each CST, a different pattern of inflammation is activated and orchestrated both by the dominant *Lactobacillus* spp. and the dominant non-*Lactobacillus* bacteria. Nugent scores (0–3) and (7–10) were confirmed in 99.3% and 89.7% cases, respectively, by molecular technique. Among Nugent scores 4–6 (partial BV), qPCR identified 46.1% of BV cases, with 37.3% of cases negative for BV and only 16.7% of partial BV (Gram stain *vs* qPCR p value = 0.0001). Among the qPCR BV cases, coinfection of bacteria involved in aerobic vaginitis and STIs was identified ($p < 0.0001$).

Discussion and Conclusions: this study contributes to the characterization of vaginal BV reshaping this concept by taking into consideration the CST classification, the local immune markers, and the immune–microbial network.

104 - Prebiotics combinations exert a Staphylococcal species-specific action balancing the skin microbiota

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Introduction. An unbalanced skin microbiota due to an increase in pathogenic *vs* commensal bacteria can be efficiently tackled by using prebiotics. The aim of this work was to identify novel prebiotics combinations able to exert a species-specific action between *S. aureus* and *S. epidermidis* strains.

Materials and Methods. First, the antimicrobial/antibiofilm effects of Xylitol-XYL and Galacto-OligoSaccharides-GOS combined with each other at different concentrations (1, 2.5, 5%) against *S. aureus* and *S. epidermidis* clinical strains were evaluated in time. Second, the most species-specific concentration was used to combine XYL with Fructo-OligoSaccharides-FOS, IsoMalto-Oligosaccharides-IMO, ArabinoGaLactan-LAG, inulin, dextran. Experiments were performed by OD_{600} detection, biomass quantification and Live/Dead staining against 4 clinical strains: *S. aureus* 815, *S. aureus* PECHA 10, *S. epidermidis* 317, *S. epidermidis* MDG1. The most performing prebiotics combinations were also evaluated in *S. aureus* and *S. epidermidis* co-colture by CFU/ml detection at 3 and 24h.

Results. The combination of 1%XYL + 1%GOS showed the best species-specific action with immediate antibacterial/antibiofilm actions against *S. aureus* strains (percentages of OD_{600}/biomass reductions up to 34.54% ± 5.35/64.68% ± 4.77) without a relevant effect on *S. epidermidis*. Among the other prebiotic formulations, 1%XYL + 1%IMO (percentages of OD_{600}/biomass reductions up to 41.28% ± 4.88/36.70% ± 10.03) or 1%LAG (percentages of OD_{600}/biomass reductions up to 38.21% ± 5.31/83.06% ± 5.11) showed antimicrobial/antibiofilm effects similar to 1%XYL + 1%GOS. For all tested formulations, a prevalent bacteriostatic effect in planktonic phase and a general reduction of *S. aureus* biofilm formation without loss of viability were recorded. The selected prebiotics combinations showed, also, a relevant specie-specific action in co-colture especially at 3h.

Discussion and Conclusions. The combinations of 1%XYL with 1%GOS or 1%IMO or 1%LAG, may help to control the balance of skin microbiota, representing good candidates for topic formulations.

69 - Is the diversity of duodenal microbiota correlated to gastric pH in functional dyspepsia?

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Introduction

Altered gut microbiota has been associated to the etiopathology of different gastrointestinal conditions, including dyspepsia. Dyspepsia is characterized by epigastric pain or burning, postprandial fullness or early satiety and most patients have no structural diseases (functional dyspepsia, FD), while others may present structural disorders like chronic atrophic gastritis (CAG). Herein, the aim of our study was to investigate the differences of the microbiota from gastric and duodenal biopsies, collected for the first time via aseptic forceps devices, and faecal samples, between patients with FD and CAG.

Materials and Methods

11 Patients with FD (ROMA III, 51 ± 4.5 years) and 20 pts with CAG (52.8 ± 2.9 years, p=NS) were enrolled in Sapienza University Gastroenterology Unit, undergoing endoscopy. From each patient, faecal samples were provided, gastric and duodenal biopsies were collected via Brisbane Aseptic Biopsy Device, and gastric pH was measured by titration. Microbiota composition was determined via 16s rDNA sequencing of the hypervariable region V3-4 on Illumina MiSeq. Alpha and beta-diversity indices and all statistical analysis were computed in Qiime2 and a $p<0.05$ was considered significant.

Results

The gastric (46 InterQuartile Range, IQR 18) and duodenal (39 IQR 34) microbiota presented significantly fewer bacterial species, and clustered separately, than the faecal microbiota (73 IQR 63, $p<0.0001$). A lower microbial diversity in the duodenal microbiota has been observed in patients with FD as compared to patients with CAG ($p<0.05$), through alpha and beta-diversity indices (Shannon's and Faith's diversity, and UniFrac analysis). By contrast, no difference was observed for the gastric and faecal microbiota. Gastric pH was significantly higher in CAG (5.9 ± 2.3) than in FD patients (2.1 ± 1.7, $p<0.00002$) and the diversity of the duodenal microbiota was directly correlated to pH ($p<0.0001$). Lastly, a strong association between two bacterial species of oral origin, *Granulicatella adiacens* and *Streptococcus salivarius*, and the patients with CAG was observed by using the ANalysis of COmposition of Microbiomes (ANCOM) and the Linear discriminant analysis with Effect Size measurement (LEfSe).

Discussion and Conclusions

Our preliminary results highlight the importance of the investigation of duodenal microbiota in patients with FD, as evidenced by a significant correlation of reduced bacterial diversity with low gastric pH in this population. On the contrary, patients with CAG, who had a high pH, showed increased diversity of the duodenal microbiota with the prevalence of oral bacterial species, suggesting reduced protective effects of the gastric barrier.

145 - Impact of gut dysbiosis in a mouse model of Parkinson disease and beneficial effects of butyrate

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1. Introduction: It has been proposed a relationship between the complexity and diversity of gut microbiota (GM) and Parkinson Disease (PD). Our preliminary data showed that antibiotic exposure was able to increase the number of contralateral amphetamine-induced rotations and reduce the striatal tyrosine hydroxylase expression in a pharmacological mouse model of PD and postbiotic treatment with sodium butyrate ameliorated PD related symptoms. These results prompted us to investigate GM composition upon indicated treatments to identify bacterial taxa potentially involved in modulation of the disease.
2. Materials and Methods: Using targeted sequencing of the 16S rRNA gene we profiled the structure of the fecal microbiota of PD mice exposed to ceftriaxone (CFX) and/or treated with sodium butyrate (BuNa). Mice were randomly divided into five groups (n=6): control mice receiving vehicle (CON); mice receiving CFX for five days (CFX); PD mice lesioned by striatal hydroxydopamine injection (6-OHDA); mice receiving CFX for five days and subsequently lesioned (6OHDA+CFX); 6OHDA mice receiving BuNa for 14 days after CFX induced dysbiosis (6OHDA+CFX+BuNa). GM was studied by sequencing of V3-V4 regions of 16S rDNA on Illumina MiSeq platform. Microbiota data analysis was conducted with QIIME2 integrated with LEFSe algorithm to identify the cohort of bacteria marking the GM of each group.
3. Results: Microbiota data analysis showed that, in addition to worsening the phenotype, CFX exposure altered the composition of GM in 6OHDA mice. 6OHDA+CFX microbiota displayed decreased alpha diversity, a different membership of bacteria in beta-diversity and a significant reduction of 11 and 14 bacterial genera compared to CON and 6OHDA mice, respectively, with *Odoribacter*, *Bacteroides*, *Prevotella*, *Defluviitalea*, *Papillibacter* and members of Lachnospiraceae and S24-7 families decreased in 6OHDA+CFX with respect to both control groups. Furthermore, BuNa treatment overturned the influence of 6OHDA and CFX by restoring to control levels the relative abundance of all the above listed bacterial genera, even if carrying a new microbial balance dominated by elevated levels of *Akkermansia muciniphyla*.
4. Discussion and Conclusions: Worsening of neuropathology of PD upon antibiotic exposure together with metatassonomic and altered profiles of GM strongly indicate a role of intestinal microbes in 6OHDA mouse model of PD. This hypothesis is corroborated by positive impact of butyrate treatment on PD related symptoms in this model, which is also accompanied by a reshaping of microbial communities. Our results strengthen the current vision that GM can be considered as potential mediator of PD symptoms and a target for their improvement.

116 - Influence of coagulase-negative staphylococci as potential probiotics on Dry Eye

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Introduction. Dry eye (DE) is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability with potential damages to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. Among the factors that cause DE, the most common is the dysfunction of the Meibomian glands that contribute to the production of the lipid component of the film. This condition alters the integrity of the ocular surface and, consequently, the microbiome. The altered microbiome exerts its pathogenetic effect in favor of a proliferation of bacteria that can produce lipase, potentiating inflammation, and triggering the innate immune response. Coagulase-negative staphylococci (CoNS) are the most frequently identified species in a healthy eye. However, *Pseudomonas*, *Acinetobacter*, *Bacillus* and *Corynebacterium* are the most frequently isolated genera in patients with DE. The objective of the study was to assess the properties of a probiotic strain of *Staphylococcus epidermidis* as a prototype for a screening study aimed at evaluating the probiotic capabilities of CoNS isolated from patients with DE. Materials and Methods. Strains: *S. epidermidis* DSM1798, *Lactobacillus reuteri* DSM20016, *Bifidobacterium longum* DSM20088, *S. aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 9027. *In vitro*, probiotic abilities have been evaluated by the following tests: aggregation, co-aggregation to pathogenic strains, biofilm production, lipase production, cytotoxicity and adhesion to corneal epithelial cells (HCE). Results. *S. epidermidis* and *B. longum* have been shown to have significant self-aggregating and co-aggregating capabilities *versus P. aeruginosa*, as well as ability to produce biofilms at 24 h. *L. reuteri* showed both significant co-aggregating capacity *vs S. aureus* and ability to produce biofilm at 48 h. The three probiotic strains produced no lipase and showed no cytotoxicity. *S. epidermidis* showed a significant ability to adhere to HCE after 24 h and 48 h of incubation. *B. longum* showed the same capacity after 48h. Discussion and conclusions. Restoring the ocular microbiota could improve the prognosis of the ocular surface involved in diseases such as DE. *S. epidermidis* probiotic has been shown not to produce lipase, to produce biofilms, to have good aggregating and self-aggregating capabilities, excellent ability to adhere to HCE and not to be cytotoxic. The results obtained with this probiotic strain encourage the continuation of the study towards the identification of CoNS isolated from patients with or without DE with probiotic abilities more specific to the ocular surface.

108 - Gut microbiota markers associated with extreme longevity in healthy Sardinian subjects

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Abstract

Introduction: Due to its impact on human metabolism and immune system, gut microbiota (GM) has been proposed as a possible determinant of healthy aging. Although growing evidence shows a progressive and adaptive remodeling of GM towards a pro- inflammatory phenotype in the aging process, in extreme longevity the dominance of bacterial taxa with an anti-inflammatory action was observed; however, to date few studies have been carried out on this aspect. The aim of the study was to analyze the GM variability of healthy Sardinian long-lived subjects (LLS), of which 17 centenarians (CENT, age= 102.18± 2.325) and 29 nonagenarians (NON, age= 93.10± 2.48) in comparison with 46 healthy younger controls (CTLs, age= 50.98± 8.312) and that of a subgroup of 8 centenarians (CPAR, age= 102± 2) compared with a paired cohort of centenarians' offspring (COFF, age= 65± 7).

Materials and Methods: Total DNA was extracted from each subject's stool sample. Bacterial load was estimate by qPCR and barcoded amplicon libraries were generated using primers targeting the V3 and V4 hypervariable region of the bacterial 16S rRNA gene. Genomic libraries were pooled and sequenced on the Illumina MiSeq platform. Operational Taxonomic Unit mapping to the Greengenes database were performed using the Quantitative Insights Into Microbial Ecology platform.

Results: Most of the significant GM alterations emerged from the comparison between LLS groups and CTLs; CENT and NON differed significantly in some bacterial taxa, but no statistically significant difference comparing CPAR and COFF was observed. The Verrucomicrobia phylum and its members Verrucomicrobiaceae and *Akkermansia* were identified as the main biomarker in CENT, together with Acidaminococcaceae (Firmicutes), Synergistetes and Euryarchaeota phyla, *Bifidobacterium* spp. (Actinobacteria) and some Bacteroidetes and Proteobacteria taxa. Actinobacteria (with related Bifidobacteriaceae and *Bifidobacterium* taxa) and Bacteroidetes phyla (with its members Bacteroidaceae and *Bacteroides*) showed strongest associations with NON and CTLs, respectively.

Discussion and Conclusions: Although the relative abundance of different pro-inflammatory microbial taxa was increased in LLS compared to CTLs, several taxa with anti- inflammatory effect were among the main biomarkers in LLS, confirming the hypothesis of a peculiar gut microbial profile of health in longevity. Our results support the hypothesis of the modulatory effect of genetics in both longevity and GM phenotype, although the CPAR and COFF cohorts need to be expanded. Further studies exploring the correlation of microbial biomarkers with significant metabolic pathways and/or with a centenarian genetic profile are needed to investigate the biological significance of these associations.

96 - Maternal anthropometric variables and clinical factors shape neonatal microbiome

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Introduction. Until recently it was believed that the fetus develops in a sterile environment and microbial colonization of the gastrointestinal tract begins at birth, through the exposure to microorganisms deriving from maternal bacteria and from environmental sources. Meconium is the first excretion product of newborn mammals, and bacterial presence in meconium samples has been recently confirmed by several studies.
While the maternal gut, oral, vaginal, and uterine microbiomes are considered key sources for meconium microbiome development, other factors are thought to influence and shape the microbiome composition during the prenatal period or during delivery in terms of both abundance and composition, such as maternal diet, maternal stress, maternal antibiotic exposure during pregnancy, delivery mode and gestational age. However, not all studies showed concordant results.
Materials and Methods The meconium microbiome composition and the predicted microbial metabolic pathways were analysed in a consecutive cohort of 96 full-term newborns. After microbial DNA extraction from meconium, the 16S gene hypervariable regions V3-V4 were sequenced by NGS. Taxonomical classification was carried out at each taxonomic level. The effect of maternal epidemiological variables on meconium alpha and beta diversity were analysed using regression analysis and PERMANOVA.
Results Meconium microbiome composition mainly included Proteobacteria (30.95%), Bacteroidetes (23.17%) and Firmicutes (17.13%), while for predicted metabolic pathways, the most abundant genes belonged to the class “metabolism”. Diversity analyses indicated a significant effect of maternal Rh factor on Shannon and Inverse Simpson indexes (p=0.045 and p=0.049 respectively) and a significant effect of delivery mode on Jaccard and Bray-Curtis dissimilarities (p=0.001), while gestational age was associated with observed richness and Shannon indexes (p=0.018 and 0.037 respectively), and Jaccard and Bray-Curtis dissimilarities (p=0.014 and 0.013 respectively).
Discussion and Conclusions Our results suggest an association between maternal Rh factor and alpha diversity, and an effect of delivery mode on beta diversity, while gestational age resulted associated with both alpha and beta diversity. Notably, since a large proportion of microbial diversity was essentially unexplained despite the use of most well-studied clinical and anthropometric variables, other factors may have a role in shaping meconium microbiome diversity. In particular, the interesting association between the genetically determined maternal Rh factor and alpha diversity indexes suggests that host genetics could have a role in newborn meconium microbiome composition.

147 - The cutaneous microbiome of the human face

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For the past 10 years, numerous microbiome studies have arisen thanks to the advent of NGS techniques allowing the precise characterization of given microbial communities. In humans, research on the cutaneous microbiome focused mainly on body niches and auto-immune or immune-mediated diseases. Much less is known about the healthy cutaneous microbiome of the human face , which prompted us to try and characterize the bacterial community that lives on our face.

This study was conducted on 118 women and 58 men of ages 19-70, living in central northern Italy and presenting no dermatological condition in the sampled area; community members were identified through 16S rRNA gene sequencing, targeting the V1-V3 hypervariable regions. Samples were collected on the subjects' right cheek, in the morning and on skin unwashed from 8-12h prior to sampling. Swabs and area-defining templates were kindly provided by Copan, while the kits for DNA extraction, PCR-mediated DNA amplification and 16S rRNA gene sequencing were purchased from Qiagen, Arrow Diagnostics and Illumina, respectively. Sequencing was performed on the Illumina MiSeq sequencer, and the resulting raw data were processed using the MicrobAT software (SmartSeq) for the primary analysis and Microbiome Analyst software https://www.microbiomeanalyst.ca) for the secondary analysis.

Over 200 samples were collected between July 2020 and April 2021, and the data was analysed following four criteria: i) sex (F=female, M=male); ii) age (under 25, 25-45, over 45); iii) season (Sp=spring, Su=summer, A=autumn, W=winter); iv) location (MI=Milan; MM=Milan metropolitan area; PO=Po River plains; MF=mountain footing). Our goal was to draw a detailed picture of the bacterial inhabitants of the normal skin, and we were able to determine certain patterns based on two endogenous (sex, age) and two exogenous (season, living area) factors.

Through this work, we gained a better understanding of the normal microbiome of the human face and how its biodiversity and taxa representation can be affected by environmental or physiological variations. Future work will focus on how the smallest fluctuations indicate or lead to dysbiosis, related whether on major actors' population or the minor taxa.

79 - Comparison of the microbiome of periodontal, peri-implant and healthy sites

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Introduction. Peri-implantitis has recently been defined as a pathological condition that occurs in peri-implant tissues and is characterized by inflammation in the peri-implant mucosa and progressive loss of supporting bone. The interaction of the oral microbiome, the host and the periodontal and peri-implant diseases is complex and is increasing, also due to the extensive use of implants. Despite the validity of dental procedures, the prognosis of peri-implantitis therapy, however, is far away from satisfactory today. Many studies, recently, have addressed the polymicrobial nature of the disease and pointed out the complex role of a dysbiotic peri-implant microbiome in the destructive tissue effects of the disease progression. This study is focused on determining the microbiome composition of healthy, periodontal and peri-implant sites using high-throughput sequencing of the 16S rRNA gene amplicon to determine a specific microbiome and identify, from a microbiological standpoint, what differences might contribute to periodontal and peri-implant diseases

Materials. A total of 10 healthy, 24 periodontitis and peri-implant sites from 24 patients were sequencing by Illumina MiSeq platform. Total DNA from paper cone was extracted and 16S rRNA gene was amplified. OTUs were picked using QIIME and analyzed by LEfSE and STAMP software.

Results

A statistically significant increase in microbial diversity across three sites was observed. The most abundant taxa in healthy sites were *Clostridiales, Bradyrhizobiaceae, Afipia, Rhodobacteraceae, Alcaligenaceae, Burkholderiaceae,Lautropia, Cardiobacterium*and*Acidovorax*, where *Lautropia* appeared as a health biomarker, whereas *Olsenella, Mongibacterium* and *Dialister* were the most discriminative for disease groups.

Conclusions

To date, the diagnosis and treatment of peri-implantitis represent an important issue because of their clinical implications. Our findings highlight a remarkable distribution of microbial populations associated with periodontal, peri-implant sites and corresponding healthy sites matching states of health and disease. In this experimental plan, diseased and healthy sites had distinct microbiological ecosystems and changed their microbial profile between health and disease in the same individual, giving new insights into the host-microorganism interactions in peri-implant disease.

65 - Metaproteomic analysis of luminal content microbiota from colon cancer patients

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1. Introduction

Recent studies have provided evidence of interactions among the gut microbiota, local host immune cells and intestinal tissues in colon carcinogenesis. However, little is known regarding possible associations between the functions exerted by the intestinal microbiota and colon cancer, particularly with respect to tumor clinical classification and lymphocyte infiltration. In addition, stool, usually employed as proxy of the gut microbiota, cannot fully represent the original complexity of colon cancer microenvironment. This pilot study was aimed at characterizing the metaproteome of tumor-associated colonic luminal contents and evaluating its potential to identify associations between gut microbial protein functions and colon cancer clinicopathological features, namely tumor stage, tumor grade and Tumor Infiltrating Lymphocytes (TILs).

2. Materials and methods

Luminal content samples were collected in operatory room before surgery from 24 colon cancer tissue specimens. After protein extraction and digestion, peptide mixtures were analyzed by high-resolution mass spectrometry. Bioinformatic analysis allowed peptide identification, label-free quantification and taxonomic/functional annotation. For each clinical variable, we identified the set of most discriminating peptides through a permutation-based sPLS regression approach. Significantly enriched taxa, functions and metabolic pathways were identified for each set of discriminating peptides, performing a sensitivity analysis considering covariate impact (age, sex and other clinical variables) and adjusting for multiple testing through a premutation-based approach.

3. Results

We identified 294, 94 and 568 microbial peptides discriminating for tumor stage, grade and TILs, respectively. Proteins produced by *Bifidobacterium* were found significantly enriched in high-stage tumors, whereas those expressed by *Bacteroides* spp. were over-represented in high-grade and TIL-negative tumor samples. Furthermore, microbial enzymes involved in tetrahydrofolate interconversion, glutamine biosynthesis and galactose catabolism were enriched in the colonic luminal metaproteome of high-stage/grade tumors.

4. Discussion and Conclusions

The metaproteomic approach used in this study has proven to be able to provide a detailed picture of the microbial and host components of the colonic luminal proteome. Moreover, promising correlations between the abundance of human and bacterial proteins and colon cancer clinicopathological features were found. Future studies with higher numbers of samples are needed to extend the investigation and confirm its biological value, as well as to validate their potential to enhance our knowledge concerning colorectal cancer progression.

114 - Distinct gut microbiota and metabolome enterotypes associated with clinical phenotypes of Parkinson's disease

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Abstract

Introduction: Parkinson's disease (PD) is a clinically heterogeneous disorder characterized by distinct clinical phenotypes associated to motor and non-motor dysfunctions. Most studies on motor deficits dichotomize PD into tremor dominant (TD) or non-tremor dominant (non-TD) phenotypes with akinetic-rigid features (AR). Different pathophysiological mechanisms may affect the onset of motor manifestations. Recent studies have suggested that gut microbes may be involved in PD pathogenesis and possibly impair motor function through microglial activation. The aim of this study was to investigate the gut microbiota and metabolome composition of PD patients in relation to TD and non-TD phenotypes.

Materials and Methods: Idiopathic PD patients (n = 56) were evaluated by the Movement Disorder Society-Unified Parkinson's Disease part III and IV and by the Non-Motor Symptom Scale, and classified into two main groups according to subtypes categories. The gut microbiota and metabolome profiles of the PD patients were determined in fecal samples using 16S next generation sequencing and gas chromatography–mass spectrometry approaches.

Results: The results revealed that the overall gut microbiota structure significantly differs between TD and non-TD subtypes, showing a reduction in diversity and richness associated to non-TD phenotypes. In particular, we found a reduction in the relative abundance of protective bacteria (i.e., *Lachnospiraceae*, *Blautia*, *Coprococcus*, *Lachnospira)*, and an increase of those correlated with pro-inflammatory activity (i.e., *Enterobacteriaceae*, *Escherichia* and *Serratia*) in association with the non-TD subtypes. Moreover, the levels of nicotinic acid, cadaverine, and glucuronic acid were altered in relation to the severity of non-tremor manifestations.

Discussion and Conclusions: Our study highlights that the gut microbiota of PD patients with TD and non-TD motor phenotypes differs in the bacterial diversity and taxonomic abundance, suggesting a possible relationship between gut dysbiosis and motor impairment. We hypothesize that the microbiota/metabolome enterotypes associated to non-TD subtypes may favor the development of a gut inflammatory milieu and gastrointestinal dysfunctions hence a more severe α-synucleinopathy. This study adds further information on PD pathogenesis and emphasizes the potential pathophysiological correlation between the gut microbiota/metabolites and PD motor subtypes.

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