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ABSTRACT BOOK

Con la sponsorizzazione non condizionante di:

Sommario

1 MICROBIOTA, MICROBIOMA, PROBIOTICI

2 - Impact of a moderately hypocaloric Mediterranean diet on the gut microbiota composition of Italian obese patients

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INTRODUCTION
Although it is known that the gut microbiota (GM) can be modulated by diet, the efficacy of specific dietary interventions in determining its composition and diversity in obese patients remains to be ascertained. The present work aims to evaluate the impact of a moderately hypocaloric Mediterranean diet on the GM of obese and overweight patients (OB).

MATERIALS AND METHODS
The GM of 23 OB patients (F/M= 20/3) was compared before (T0) and after 3 months (T3) of the nutritional intervention (NI). At baseline, a group of 46 normal-weight healthy subjects (NW) was used as control. Barcoded amplicon libraries for the bacterial community analysis were generated using primers targeting the V3 and V4 hypervariable regions of the bacterial 16S rRNA gene and Nextera XT index kit (Illumina, inc.). Samples were sequenced and analyzed with Illumina MiSeq platform and the 16S metagenomics app and the MiSeq Reporter software.

RESULTS
At baseline, the GM characterization confirmed the typical obesity-associated dysbiosis. After 3 months of NI, patients presented a statistically significant reduction of the body weight and fat mass, along with changes in the relative abundance of many microbial patterns. In fact, we observed an increased abundance in several Bacteroidetes taxa (i.e. Sphingobacteriaceae, *Sphingobacterium*, *Bacteroides* spp., *Prevotella stercorea*) and depletion of many Firmicutes taxa (i.e. Lachnospiraceae members, Ruminococcaceae and Ruminococcus, Veillonellaceae, *Catenibacterium*, *Megamonas*). In addition, the phylum Proteobacteria showed an increased abundance, while the genus *Sutterella*, within the same phylum, decreased after the intervention. Worth mentioning, the GM of OB patients did not longer segregate from that of NW controls. Metabolic pathways, predicted by bioinformatic analyses, showed a decrease in membrane transport and cell motility after NI.

DISCUSSION AND CONCLUSIONS
This study confirms the pathological role of an increase in Firmicutes and depletion in Bacteroidetes in obese patients and the potential role of other taxa linking obesity and intestine. Moreover, findings from the present study underline the potential benefit of a moderately restrictive nutritional approach based on the Mediterranean diet in counteracting the gut dysbiosis, commonly observed in obese and overweight patients.

4 - In vitro evaluation of the antagonistic properties of Lactobacillus paracasei LA802 against pathogens involved in upper respiratory tract infections

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Introduction: Accumulated evidence suggests that probiotics have beneficial effects in upper respiratory tract infections (URTIs) by decreasing their incidence, duration and severity of symptoms. The objective of this study was to evaluate *in vitro* the ability of *Lactobacillus paracasei* LA802 to antagonize bacterial pathogens frequently involved in URTIs in comparison with two reference strains, *Lactobacillus rhamnosus* GG and *Streptococcus salivarius* K12.

Materials and Methods: The antagonistic activity of the three potential probiotic strains was evaluated against five bacterial species (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*) using three agar diffusion methods: spot-agar test, drop diffusion and well-diffusion. Their ability to interfere with the adhesion of *H. influenza*e (ATCC9007) and *S. pneumoniae* (ATCC700677) was performed *in vitro.* Polystyrene surfaces coated with collagen type I were used to simulate the conditions of the upper respiratory tract. In competitive adhesion assays, the probiotic strains were introduced simultaneously with the pathogenic bacteria in wells and incubated for 2 hours. In exclusion assays, the probiotic strains were incubated first for 2 hours, then washed away and incubated with pathogenic bacteria.

Results: *L. paracasei* LA802 exhibited antagonistic activity against all the pathogenic strains tested. On the contrary, the two reference strains were not able to inhibit the growth of *S. aureus*. The results obtained with the three agar diffusion methods suggest that their antagonistic effects involve different mechanisms of action. In competitive adhesion assays, significant differences were observed among the strains tested against the two pathogens. *L. paracasei* LA802 induced a 50% decrease in the amount of *S. pneumoniae* adhering to type I collagen (not significant) and a significant 55% decrease in the amount of adhered cells of *H. influenzae* ($p<0.01$). A significant reduction of pathogen adhesion was also observed with *L. rhamnosus* GG whereas *S. salivarius* K12 had no significant effect. In exclusion assays, *L. rhamnosus* GG and *S. salivarius* K12 slightly reduced the adhesion of *S. pneumonia* (*L. paracasei* had no effect) whereas the three strains decreased that of *H. influenzae*, with a greater effect of *L. paracasei* LA802 (52% decrease).

Discussion and Conclusions: This study demonstrated the ability of *L. paracasei* LA802 to inhibit the growth of pathogens involved in URTIs and to limit pathogen adhesion in particular that of *H. influenzae*. By interfering with pathogens during primary adhesion, the strain may be of interest in the prevention of bacterial infections and superinfections of the respiratory tract.

9 - Fecal microbiota signatures in celiac disease patients with poly-autoimmunity

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Introduction
To date, reliable tests enabling the identification of celiac disease (CD) patients at a greater risk of developing poly-autoimmune diseases are not yet available. A considerable amount of studies has demonstrated the association between autoimmunity and fecal microbial signature, yet investigations on gut microbiota (GM) and poly-autoimmunity are still limited. In order to discover the presence of any modifications concerning GM which could be related to poly-autoimmunity, we aimed to identify non-invasive microbial biomarkers, useful to implement diagnosis of immune disorders.

Materials and Methods
Twenty CD patients with poly-autoimmunity (cases) and 30 matched subjects affected exclusively by CD (controls) were selected. All patients followed a varied gluten-free diet for at least 1 year. Fecal microbiota composition was characterized using bacterial 16S ribosomal RNA gene sequencing. ELISA tests were performed on serum and stool samples in order to examine gut permeability (lipopolysaccharide and its binding protein, core endotoxin, and zonulin) and intestinal inflammation markers (fecal calprotectin, fecal B-cell activating factor (f-BAFF), and intestinal fatty acid binding protein).

Results
Significant differences in GM composition between CD patients with and without poly-autoimmune disease were found using the edgeR algorithm. Spearman correlations between GM and clinical, demographic, and anthropometric data were also examined. A significant reduction of *Bacteroides*, *Ruminococcus*, and *Veillonella* abundances was found in CD patients with poly-autoimmunity compared to the controls. *Bifidobacterium* was specifically reduced in CD patients with Hashimoto's thyroiditis and its abundance correlated negatively with abdominal circumference values in patients affected exclusively by CD. In addition, the duration of CD correlated with the abundance of Firmicutes (negatively) and *Odoribacter* (positively), whereas the abundance of *Desulfovibrionaceae* positively correlated with the duration of poly-autoimmunity. Serum biomarkers did not differ significantly between cases and controls.

Discussion and Conclusions
This study provides supportive evidence that specific variations of gut microbial taxa occur in CD patients with poly-autoimmune diseases. In particular, the reduction of *Bacteroides*, *Bifidobacterium*, *Veillonella*, and *Ruminococcus* might represent a potential biomarker of poly-autoimmunity. These findings open the way to future validation studies on larger cohorts, which might in turn lead to promising knowledge-based diagnostic applications and novel therapeutic approaches.

13 - Evaluation of viable microbial community composition of Agnano Thermal Spring Water

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Introduction: The Agnano Thermal Spring Water (ATSW) is mainly a salso-bromo-iodic-bicarbonate-alkaline-sulphurous water. The benefits of thermal water in the treatment of various diseases have been known since ancient times. Thermal water has therapeutic functions and it is possible to use it in the treatment of various dysfunctions, most of these beneficial effects depend on its chemical and physical properties. However, some effects, such as improved cell proliferation or anti-inflammatory properties, may not be fully explained only by the mineral composition. For this reason, the aim of the present study is the microbiological characterization of ATSW through the use of two distinct methods in order to determine the total viable bacterial community as a non-pathogenic bacterial populations that may play an active role in various processes in the ecological and biological fields.

Materials and Methods: ATSW was collected in two different seasons of the year; in winter (January 2019) and autumn (October 2019) with an aseptic procedure. In order to increase the yield for the microbiological characterization of ATSW, the analyzes were carried out using two procedures: filtration and enrichment. **Results:** The microbiological analyzes for the two samplings have highlighted the identification of bacterial species belonging mainly to three different phyla: Actinobacteria, Firmicutes and Proteobacteria, with an abundance of species belonging to the Phylum Firmicutes. Moreover, ATSW is a rich source of minerals. About 31 minerals have been identified of which 17 are found as major ions involved in skin barrier recovery.

Discussion and Conclusions: The obtained data show that the viable microbial community recovered from these two collections of the ATSW is mainly composed of bacterial species belonging to three different Phyla and that these populations can undergo seasonal variations. Recent data demonstrated that, salso-bromo-iodine water showed beneficial activities on mucous-secretory disorders, improving the relationship between the mucous-protein complexes and the water. Therefore, the ATSW for its mineral composition and microbial diversity exhibits both prebiotic and probiotic characteristic, thus a further study will be set up for the evaluation of anti-inflammatory and regenerative properties of ATSW in an *in vitro* system.

25 - Microbial profile shift and miRNAs circulating in the saliva: what is their clinical correlation?

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Introduction: Several pathological conditions, as well as neuro-psychiatric diseases, may modify salivary molecules including metabolites, proteins, RNAs and bacterial populations. Autism Spectrum Disorder (ASD), a complex neurodevelopmental disorder whose etiopathogenesis is still unclear, is believed to be the complex result of a combination of genetic, epigenetic and environmental factors. Immune dysregulation and gastrointestinal abnormalities are of particular interest in the light of several papers reporting ASD-associated disturbances. Many studies have reported that in ASD patients gut microbiota dysbiosis could play a key role in the alterations of brain structure and function development because of interactions between the Central Nervous System (CNS) and the gut microbiota; the so-called gut-brain axis. The aim of this study was to combine the alterations of the salivary microbiome and miRNA expression profiles in ASD and healthy subjects and their association with neuropsychological parameters to determine new biomarkers of ASD.

Materials and methods: In this experimental plan, were evaluated changes in the microbial composition of the salivary microbiome in 53 ASD and 27 HE (healthy) samples, sequenced on the Illumina MiSeq platform. To profile the circulating miRNA expression from saliva, NanoString nCounter system assays were performed using the NanoString platform.

Results: The microbial profile by 16SrRNA sequencing analysis of ASD patients and HE subjects revealed statistically significant differences of abundance at the genus and species levels. In particular, *Rothia*, *Filifactor*, *Actinobacillus*, *Weeksellaceae*, *Ralstonia*, *Pasteurellaceae* and *Aggregatibacter* increased their abundance rates in the saliva of ASD patients, while *Tannerella*, *Moryella* and TM7-3 decreased. In addition, 5 salivary miRNAs were statistically altered in ASD patients compared to HEs. Variations of both miRNAs and microbes were statistically correlated to different neuropsychological scores related to anomalies in social interaction and communication. Interestingly, we also found a negative correlation between salivary miR-141-3p expression and *Tannerella* abundance.

Discussion and Conclusions: In our study, we demonstrated that miRNA and microbiome dysregulations found in the saliva of ASD children are associated with cognitive impairment of the subjects and a potential cross-talking between circulating miRNAs and resident bacteria alterations could exist. Moreover, these findings could pave the way to new potential tools for molecular diagnosis of ASD. We would like to thank the "PIACERI", Department Research Plan of University of Catania 2020 (2nd line of intervention).

27 - Defining the oral microbiome by Whole-Genome Sequencing and resistome analysis: the complexity of the healthy picture.

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Introduction

The microbiome of the oral cavity is the second-largest and diverse microbiota after the gut, harboring over 700 species of bacteria and including also fungi, viruses, and protozoa. With its diverse niches, the oral cavity is a very complex environment, where different microbes preferentially colonize different habitats. Recent data indicate that the oral microbiome has essential functions in maintaining oral and systemic health, and the emergence of 16S rRNA gene next-generation sequencing (NGS) has greatly contributed to revealing the complexity of its bacterial component. However, a detailed site-specific map of oral microorganisms (including also eukaryotes and viruses) and their relative abundance is still missing. Here, we aimed to obtain a comprehensive view of the healthy oral microbiome (HOM), including its drug-resistance features.

Materials and Methods

The oral microbiome of 20 healthy young subjects (mean age 24.7 years, range 21-30) was analyzed by whole-genome sequencing (WGS) and real-time quantitative PCR microarray. Sampled oral micro-habitat included tongue dorsum, hard palate, buccal mucosa, keratinized gingiva, supragingival and subgingival plaque, and saliva with or without rinsing.

Results

Each sampled oral niche evidenced a different microbial community, including bacteria, fungi, and viruses. Alpha-diversity evidenced significant differences among the different sampled sites ($p<0.0001$) but not among the enrolled subjects ($p=0.876$), strengthening the notion of a recognizable HOM. Of note, oral rinse microbiome was more representative of the whole site-specific microbiomes, compared with that of saliva. Interestingly, HOM resistome included highly prevalent genes conferring resistance to macrolide, lincosamides, streptogramin, and tetracycline.

Discussion and Conclusions

The data obtained in 20 subjects by WGS and microarray analysis provide for the first time a comprehensive view of HOM and its resistome, contributing to a deeper understanding of the composition of oral microbiome in the healthy subject, and providing an important reference for future studies, allowing to identify microbial signatures related to functional and metabolic alterations associated with diseases, potentially useful for targeted therapies and precision medicine.

32 - Metaproteomics of luminal contents reveals microbial and human functions associated with colon cancer clinicopathological features

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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. The aim of this pilot study is to characterize the metaproteome tumor-associated colonic luminal contents, and to evaluate its possible associations with cancer clinicopathological features.

Materials and Methods: DNA and proteins were extracted from luminal content samples, collected during surgery from 24 patients with colon cancer. Proteins were analyzed by shotgun metaproteomics, and mass spectrometry data were subjected to bioinformatic analysis for database identification, label-free quantification and taxonomic/functional annotation. A DNA pool was prepared to carry out a shotgun sequencing of the whole gut metagenome. The collection of metagenomic sequences, a public human gut metagenome dataset, and a human proteome database were used to perform peptide identification using Proteome Discoverer. All clinical-pathologic data were available from medicals records for all patients.

Results: Metaproteome abundance data (taxa, functions and taxon-specific functions) were associated with clinical metadata (tumor site, stage and grade, plus the percentage of tumor-infiltrating lymphocytes). Numerous human proteins showed abundance variations clearly consistent with previous knowledge about colon cancer biology, providing evidence of the efficiency and reliability of the analytical approach used. Several microbial functions were also found to change in abundance based on tumor clinicopathological features

Discussion and Conclusions: The analytical and bioinformatic 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 will be needed to confirm the biological value of these data, as well as to validate their potential to enhance our knowledge concerning colorectal cancer progression.

34 - Dysbiosis of the gut microbiota in patients with levothyroxine malabsorption.

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In recent years, the gut microbiota, essential for ensuring digestive and immunological homeostasis, has been involved in the development of several gastrointestinal disorders, including inflammatory bowel diseases and metabolic disorders. Moreover, it has been reported the crucial role of the intestinal tract in the metabolism of nutrients, drugs and hormones, such as, in particular, iodothyronines as well as micronutrients involved in thyroid homeostasis. On this regard, it has been suggested that alterations of the intestinal microbiota may influence the pathways of thyroxine metabolism and its intestinal absorption, as evidenced by an increased need of oral thyroxine in patients with gastrointestinal disorders. The investigation of microbial profiles associated to the increased need for oral thyroxine is an intriguing issue; therefore, here we present a pilot study aiming to characterize gastrointestinal microbiota in patients with Hashimoto thyroiditis and altered levothyroxine (L-T4) absorption. Patients affected by Hashimoto thyroiditis with normal L-T4 absorption were also enrolled as control group.

Duodenal biopsies and stool samples were collected from each patient at the Gastroenterology Unit, Department of Internal Medicine and Medical Specialties, General Hospital "Umberto I", and analyzed via the metagenomic analysis of the hypervariable region v3/4 of the 16S rRNA gene (Illumina MiSeq, USA). The resulting sequencing data were investigated via complex statistical approaches (alpha and beta diversity indices and correlation analysis).

Our results showed a higher biodiversity and a different composition of the gut microbiota in patients with L-T4 malabsorption as compared to the controls. Specifically, the weighted-UniFrac analysis, a measure of beta diversity, resulted in a significant separation of the two groups ($p<0.01$). An increase in the phylum Bacteroidetes (34.6% vs 14.2%), as well as a decrease in the phylum Firmicutes (55.2% vs 78.7%) were also observed in L-T4 malabsorbing patients as compared to controls. By contrast, no difference was evidenced in the duodenal microbiota between the two groups.

Overall, our preliminary results suggest that alterations in the gut microbiota, such as increased biodiversity and a decrease in the number of Firmicutes, generally associated to the protective function of the intestinal barrier, may be related to abnormalities in levothyroxine absorption, potentially accounting, thus, for treatment-refractory hypothyroidism.

43 - Antibiotic resistance genes of the new Bifidobacterium asteroides BEBIF 17 strain isolated from honeybees' gut

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Introduction.
Bifidobacteria are known as health-promoting microorganisms. This genus showed a widespread distribution in a large variety of hosts: they are commensal of the human gut and inhabitant of the gastrointestinal tract of various animals and insects. The bifidobacteria are used as bioactive components in pharma products, dairy products and in food supplements. The *Bifidobacterium asteroides* specie is commonly present in honeybees gut and show particular features with respect other *Bifidobacterium* species. Genome analysis on strains belonging to this species suggest that it is an ancestor of the genus *Bifidobacterium*. The aim of the study was to investigate the antibiotic resistance profile of a new *Bifidobacterium asteroides* BEBIF 17 strain isolated from honeybees gut.

Materials and Methods.
The *Bifidobacterium asteroides* BEBIF 17 strain, previously characterized for functional properties, was subjected to total genomic DNA isolation using a specific kit (MACHEREY-NAGEL GmbH & Co. KG, Germany), following the manufacturer instructions. Multiplex PCR were carried out for detection of: glycopeptide resistance genes (*vanA*, *vanB*, *vanC-1*, *vanC-2*, *vanD*, *vanE*, *vanG*) using the method described by Depardieu et al. (2004); tetracycline resistant genes (*tet-M*, *tet-L* and *tetO*) using the method described by Ng et al. (2001). The detection of virulence genes (*asa1*, *gelE*, *cylA*, *esp*, *hyl*) was performed using the method described by Vankerckhoven et al. (2004). Genome sequencing of the new *Bifidobacterium asteroides* BEBIF 17 strain was performed as described by Inturri et al. (2016) afterwards, the genome analysis was perfomed on the basis of Blastp and Blastn tools.

Results. The new *Bifidobacterium asteroides* BEBIF 17 did not show genes encoding for glycopeptide and tetracycline resistance as well as virulence genes. Moreover, the genome analysis showed a DNA of 2186076 number of bases with 1695 predicted ORFs and an average GC percentage of 60.38.

Discussion and Conclusions. Data of the present study, confirmed at strain level, showed that the new isolate from honeybees' gut is a promising probiotic candidate suitable as bioactive components in pharmaceutical products, dairy products and in food supplements.

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67 - Probiotic properties of B. clausii strains

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Introduction: Desirable properties of orally administered probiotics include the ability to survive and multiply in the presence of bile, adhesion to the host intestinal mucus layer, and production of beneficial enzymes and vitamins. Bacteria of the *Bacillus* genus are frequently used in probiotic formulations for their antimicrobial and immunomodulatory activities and for the possibility to be administered as spores. This research aimed at evaluating survival rate and growth in simulated intestinal fluid, adhesion to mucins, and production of vitamin B_2 (VB_2), catalase (CAT), superoxide-dismutase (SOD), beta-galactosidase, and D-lactic acid by *Bacillus clausii* strains contained in a probiotic formulation.

Materials and Methods: Four *B. clausii* strains and a commercial formulation containing *B. clausii* spores were used. To evaluate microbial survival and replication in simulated intestinal fluid, the spore suspension and the *B. clausii* strains were inoculated in a pancreatin-bile salts solution for up to 8 hours and the number of viable cells quantified by plating at different time points. The ability to adhere to mucins was tested in aerobic and anaerobic conditions. The production of CAT, SOD, and D-lactate was evaluated by colorimetric commercial kits. The production of beta-galactosidase was evaluated on solid media and by the quantitative beta-galactosidase assay. The secretion of VB_2 was quantified by using an enzyme-linked immunosorbent assay kit for VB_2.

Results: Our data indicate that *B. clausii* survives in simulated intestinal fluid for at least 8 hours and adheres to mucins, thus suggesting its ability to persist and multiply in the human gut. The finding that *B. clausii* strains produce CAT, SOD and beta-galactosidase highlights their potential contribution in counteracting oxidative damage and in promoting lactose degradation. In addition, the analyzed *B. clausii* strains were found to be able to actively secrete VB_2 but not D-lactate. This evidence suggests that they can be administered for ameliorating host deficiency in VB_2, excluding any risk for subjects potentially prone to develop D-lactic acidosis.

Discussion and Conclusions: Bile resistance, adherence to the intestinal mucosa, and production of beneficial molecules are claimed key properties of orally administered probiotics. *B. clausii* demonstrates to fully satisfy these requirements, imposing itself in the probiotic scenario as novel approach to improve clinical conditions, such as inflammatory bowel diseases and lactose intolerance. In conclusion, this study highlights *in vitro* probiotic properties of *B. clausii*, which represent a pivotal step to understand the *in vivo* efficacy of this orally administered probiotic organism.

113 – A new topical foam for the prevention of skin dysbiosis that precedes the occurrence of pressure ulcers: preliminary data on the microbial skin community variation

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Introduction: skin is home to millions of bacteria, fungi and viruses that constitute the skin microbiota. The most commonly represented epidermal bacterial phyla are *Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes*. The microbial composition of human skin is not static and variations in the abundance of some species are related to skin diseases. The three main phyla identified in pressure ulcers are similar to those of healthy diners (e.g. *Firmicutes, Proteobacteria* and *Actinobacteria*). A new topical foam manufactured by Aileens pharma s.r.l. was tested in patients hospitalized without sacral injury and was examined the variation of the skin microbiome to determine whether this product could prevent the occurrence pressure ulcers.
Materials and Methods: seventeen patients with following inclusion criteria, were enrolled in this study: BMI between 18.5 and 24.9; Age >65 conscious; Braden's Scale Score <16; Norton's Scale Score <14. The patients included in the study at the time of recruitment had no sacral pressure lesions. The skin microbiota in two adjacent sacral areas was sampled at day 0 zero by a microfibre swab Enat (Copan) after cleaning the skin, then in the same area was applied a topical foam for the 15 consecutive days once a day after cleansing the skin. On the sixteen-day, a new sampling was performed.

Results: none of the patients treated with foam reported adverse events or voluntarily abandoned the study. Nevertheless, there were 2 drop-outs on days 9 and 3 as patients died due to the worsening of their disease. Among the remaining patients, no one developed signs of ulcers (or even slight redness) in the lumbosacral tract (treated area). In the follow-up, one patient developed a sacral sore 15 days after discontinuation of treatment, while in all other patients no lesion development was recorded even one month after discontinuation of foam. To examine potential differences in the relative abundance of the microbial composition of the skin between 1st and 2nd sampling all OTUs that were taxonomically assigned to the same group were combined. *Firmicutes, Actinobacteria e Proteobacteria* are the most abundant phyla in both isolation and show small variations in relative abundance between the two samples.

Discussion and Conclusions: in view of the impact of skin bacteria on the development of skin disorders as highlighted above, cutaneous microbiota differences may significantly contribute to the risk of developing pressure ulcers. Such potential differences may offer a new way to identify patients at increased risk for pressure ulcers and can lead to new preventive measures based on modulation of the microbiota as well. These preliminary data would suggest that a new topical foam through modification of the skin microbiota can be used as an adjuvant in the prevention of pressure ulcers.

123 - Galleria mellonella oral administration in vivo model to evaluate the probiotic potential of two Lactobacillus plantarum strains.

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Introduction: intestinal dysbiosis is renowned to contribute to the pathogenesis of inflammatory bowel diseases, and it is also involved in several pathologies. The administration of probiotics has shown promising results for prevention and treatment of dysbiosis. In fact, their action takes place at different levels, including competition with enteropathogens for intestinal epithelial cell adhesion sites. This work aims at evaluating the adhesion ability of two potential probiotic strains of *Lactobacillus plantarum* (ATCC 14917 and ATCC BAA-793), now *Lactiplantibacillus plantarum*. This study estimated the *in vitro* adhesion on the Caco-2 and HT-29 cell lines (human colon adenocarcinoma), and it assessed the *in vivo* permanence in the gut of *Galleria mellonella* an innovative model for probiotic screening.

Materials and Methods: to evaluate the *in vitro* adhesion, serial dilutions of each *L. plantarum* strain were inoculated on the cell lines Caco-2 and HT-29 and non-adherent bacteria have been removed with Dulbecco's phosphate-buffered saline (DPBS). In order, to enumerate the adherent bacteria, cells lysates have been serially diluted, plated and counted on De Man, Rogosa and Sharpe (MRS) agar. To evaluate *in vivo* permanence in *G. mellonella's* gut after gavage administration of *L. plantarum* suspensions, microbial strains isolated from *larvae's* gut were subjected to phenotypic and molecular analysis to identify the specific 16S rRNA gene sequences of *L. plantarum*.

Results: the comparison of data obtained from the *in vitro* and *in vivo* assays showed that the adhesion trend is comparable between the two models tested and, in both cases, it appears to be influenced by the bacterial concentration. This work demonstrated that the adhesion rate of the *L. plantarum* strains is generally higher on HT-29 than on Caco-2. In particular, the BAA-793 strain, isolated from human saliva, has a better adhesion performance than the ATCC 14917 food strain, both *in vitro* and *in vivo*. These results suggest a possible adaptation of this strain to its biological niche. Furthermore, the evaluation of the colonization of *G. mellonella* gut by *L. plantarum* strains, after 0 and 24 hours from gavage showed the absence of *L. plantarum* in the control group *larvae's* gut. On the other hand, the bacilliform strains isolated in the study group were phenotypically and genetically identified as *L. plantarum*.

Discussion and Conclusions: the data obtained in this preliminary study showed that *L. plantarum* can colonize *G. mellonella's* gut resulting to be a potential probiotic. This pilot study demonstrated how the *G.mellonella* oral administration model can be a successful tool for a preliminary evaluation of new potentially probiotic strains.

133 - Gut-heart axis crosstalk during heart failure: a comprehensive analysis of gut microbiota composition, intestinal barrier integrity, intestinal and systemic inflammation in mice undergoing pressure ov

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Introduction: a mutual gut-heart crosstalk has been recently proposed in heart failure (HF). Reduced cardiac output, increased tissue congestion and peripheral vasoconstriction in HF could support gut microbiota re-assortment together with disruption of intestinal barrier functions and, in turn, promote systemic inflammation with direct impact on development and progression of cardiac dysfunction. Aim of the study was to investigate gut-heart crosstalk in a mouse model of pressure overload-induced cardiac hypertrophy and heart failure induced by Transverse Aortic Constriction (TAC). The effects of TAC procedure were evaluated on gut microbiota, gut barrier integrity, intestinal and serum cytokines, serum endotoxin levels in C57BL/6 mice. Additionally, postbiotic treatment was attempted to explore possible effects on intestinal barrier integrity and inflammation levels related to TAC induced phenotype.

Material and Methods: abdominal aortic blood flow was recorded after 1, 3, 7 and 28 days after sham or TAC surgery by Pulsed Doppler. After 1w or 4w, mice were anesthetized, cardiac function and transverse aortic pressure gradients were recorded by M-Mode and Doppler echocardiography, respectively. Fecal microbiota was analyzed by high-throughput sequencing targeting the V3–V4 region of the 16S rDNA. Data were analyzed with QIIME and LDA Effect Size analysis. After sacrifice, colon samples were used to perform mRNA analysis of IL-10, zonuline-1 and occludin. IL-1α, IL-10, IL-6, TNFα and LPS levels were analyzed in serum by ELISA.

Results: Compared to sham-operated animals, TAC clearly induced weakening of intestinal barrier integrity, long-lasting decrease of colon anti-inflammatory cytokine levels, increases of serum levels of bacterial lipopolysaccharide and proinflammatory cytokines. Interestingly, TAC procedure also affects microbiota by changing the relative abundance of bacterial genera. The increase of lactate-producing bacteria (*T. sanguinis* and *L. frumenti*) co-occurred with the depletion of butyrate-producing bacteria (genus *Oscillospira*, taxonomically classified as *P. capillosus* and *F. plautii*). Attempt to use a postbiotic, namely butyrate, a potent support of epithelial health and intestinal repair,

failed to restore TAC-induced phenotype possibly because of low dosage and short time course treatment used in experimental design. Further attempts are under evaluation with other modulators of microbiota and gut integrity.

Discussion and Conclusions: The results suggest that gut modifications are an important element to be considered in the development and progression of cardiac dysfunction and TAC mice as a valuable tool to establish the importance of gut barrier function and microbiota composition in HF.

137 - How Lactobacillus plantarum shapes its metabolism under contrasting ecosystems

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Introduction

Lactobacillus plantarum possess the largest genome within the entire genus group because it did not undergo genome reduction strategy due to niche adaptation. This high genomic diversity imparts metabolic flexibility that confers *L. plantarum* the typical nomadic lifestyle. Consequently, *L. plantarum* genomes do not cluster according to the source of isolation but their capacity to respond to stressors present in various ecosystems remains unclear. This study aims to characterize the ability of two *L. plantarum* strains isolated from cheese and bees' gastrointestinal tract (GIT) to switch their metabolism under dairy, bees' GIT and pineapple ecosystems compared to MRS standard culture media.

Materials and methods

Phenotype switching, a novel phenomics approach using Omnilog platform, was applied to determine the effect of environmental conditions from pineapple and dairy ecosystems to a standard rich medium (MRS) on the metabolism of BEE1ST and CB5 strains. Metabolic parameters were determined using our deposited Micro4Food pipeline. Signal decomposition and a Bayesian approach was used to further estimate the effect of the environmental stressors and strain inherent genotype on the metabolism dynamics.

Results

Clustering of *L. plantarum* phenotypes was mainly ecosystem-dependent and not by strain genotypic traits. Strains undertook similar metabolic strategies under the same environmental stress but the ability to perform a metabolic switch was strain dependent. BEE1ST had a higher basal metabolism but CB5 possessed the greatest ability to perform a metabolic switch when cultured on pineapple or cheese model medias.

Discussion and Conclusions

These results remark *L. plantarum* retained functional diversity as a paradigm of nomadic lifestyle. However, metabolic plasticity differences among strains were highlighted. The *in vivo* phenome investigation will enable a better understanding of *L. plantarum* diversity, further genomics and transcriptomics will serve to deepen in the underlaying mechanisms of differences within strain metabolic.

2 ANTIMICROBICO-RESISTENZA

3 - Isolation of Staphylococcus microti strains from buffalo milk

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Introduction - The Mediterranean buffalo (Bubalis bubalis) is a large bovid, widely spread throughout Southern Italy. Particular value has buffalo milk production and mastitis represents the most expensive disease. For this reason, it is important to early diagnose clinical and sub-clinical mastitis, in order to prevent economic losses.
Staphylococci are the major bacterial agents causing mastitis. Precisely, staphylococci coagulase-positive (CoPS) are the main causative pathogens of sub-clinical and clinical mastitis, and they are considered to be more virulent than coagulase-negative staphylococci (CoNS) that, in comparison, appear to be highly contagious and numerous. Up to now, more than 45 CoNS recognized species have been described and among them Staphylococcus microti is a novel CoNS species. Materials and

Methods - The research was conducted on a buffalo farm in Battipaglia (SA), Italy, housing 100 lactating buffaloes. Milk samples were collected after owner permission. The animals chosen for sampling were in the lactation period, aging from 2 to 5 years, did not receive any antibiotic treatment within one month. Milk samples were checked for somatic cell count (SCC) as well as for microbiological culture tests using different media. Then, the isolated stains were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Isolates were analyzed by Kirby-Bauer disk diffusion susceptibility method against 14 different antimicrobials.

Results - Some milk samples (18.2%) showed SCC value >200.000 cell/mL. The standard microbiological culture of 44 quarter milk samples and the MALDI-TOF-MS procedure permitted the identification of 25/93 (27%) Staphylococcus spp strains. As a result of coagulase test, 2/25 (8%) isolates were CoPS and 23/25 (92%) were CoNS, respectively. Among CoNS, 10 S. microti, 2 S. simulans 2 S. sciuri, 2 S. haemolyticus, 2 S. saprophyticus, 1 S. chromogenes, 1 S. pasteuri, and 3 S. epidermidis, were identified. S. microti, S. simulans and S. sciuri were the isolates associated to intramammary infections with an elevate SCC value. High rates of resistance to tested antimicrobial agents; particularly, a resistance rate of 100% was found against tetracycline and oxytetracycline.

Discussion and Conclusions - The relevance of different bacterial pathogens in mastitis has been known for a long time. However, the CoNS as mastitis-causing agents could not be neglected and S. microti isolates could represent a novel staphylococcal species associated with a status of sub-clinical mastitis in buffaloes. Moreover, these isolates could serve as reservoirs for the transmission and spread of antibiotic resistant determinants within the farm environment.

7 - Antimicrobial efficacy of essential oils against pathogens isolates from cystic fibrosis patients by using a machine learning analysis

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Introduction: Recurrent and chronic respiratory tract infections in cystic fibrosis (CF) patients result in progressive lung damage and represent the primary cause of morbidity and mortality. *S. aureus* is one of the earliest bacteria colonizing lung airways in CF infants and children. After early adolescence patients are chronically infected with Gram-negative non-fermenting bacteria, and *P. aeruginosa* is the most relevant and recurring.
Since intensive use of antimicrobial drugs inevitably leads to antibiotic resistance, new antimicrobials should be identified to overcome antibiotic resistance in these patients.
Recently interesting data were reported in literature on natural derived compounds that inhibit bacterial growth of *S. aureus* and *P. aeruginosa* in vitro. Essential oils (EOs), among these, seem the most promising.
In this work is reported the antibiofilm activity of 61 EOs against a panel of selected clinical strains isolated from CF patients.

Materials and methods: Machine learning clusterization algorithms were applied to pick-up few representative bacterial strains in a panel of 40 clinical isolates: 6 representative for *P. aeruginosa* and 3 for *S. aureus*. As controls, reference ATCC strains were used.
The chemical composition of each EO was assessed by GC analysis. The action of each EO on biofilm formation was assessed by crystal violet staining.

Results: Bacterial strains were characterized for their ability to form biofilm. It is worth to note that each EO had a specific effect on biofilm formation, likely depending on its characteristic and unique composition previously chemically analyzed (quantitative and qualitative analysis).
Arbitrarily, 3 levels of biofilm inhibition were considered to qualitatively cluster the EOs potencies: strong biofilm inhibition in the range 0-40% of residual biofilm, mild inhibition in the range 40-80% and no biofilm inhibition over 80% of residual biofilm, respectively. In some cases an increase of biofilm formation was highlighted after the treatment. Most of EOs analyzed was able to destabilize biofilm structure without affect bacterial viability.

Discussion and Conclusions: Many EOs inhibit biofilm formation in selected strains of *S. aureus* and *P. aeruginosa*. Their clinical use can be recommended since no evidence of resistance to EOs has yet been described.

14 - Unraveling Colistin Resistance Diversity in clinical Acinetobacter baumannii: in-depth analysis of COL-R Strain-Profiling and Genomics

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Introduction

Although the increased use of colistin (COL) for the therapy of difficult to treat Gram-negative bacteria can promote the emergence of COL-resistant (COL^R) strains, the adaptation of the bacterial species to the colistin pressure need in-depth investigation. Our study focused the strain-profiling and genomics of colistin resistance (COL-R) diversity existing in 9 clinical Extensively Drug-Resistant (XDR) *Acinetobacter baumannii* (*Ab*).

Materials and Methods

COL-susceptibility was determined by repeated (10 times) Minimum Inhibitory Concentrations (MIC). COL-R inductions were performed using up to COL 2 mg/L (resistance cut-off). Population analysis profiles (PAP) were performed to investigate the presence of COL^R subpopulations. Whole Genome Sequencing was performed by Illumina Mi-Seq and the genomic epidemiology analysed by bioinformatic tools. The Variant Calling was used to identify genomic SNPs and *pmr*BCA, *lpx*D/A/C SNPs on *Ab* ACICU as RefGenome.

Results

In-*depth* analysis on COL-R strain-profiling displayed stable and reproducible MIC values (Full-Resistance) in 3 *Ab* strains, whilst 6 strains revealed unstable and variable MICs. Among these unstable phenotypes, COL-R induction revealed an inducible COL-R phenotype in 2 *Ab* strains (Adaptative-Resistance).

PAP analysis evidenced different COL^R subpopulations in the remaining *Ab* strains. In details, 3 *Ab* strains showed phenotype defined as "Heterogeneous-Resistance", in which on ≥32 mg/L COL agar-plates coexisted two morphologically different colony variants having two or more-fold COL MIC variations; 1 *Ab* strains exhibited a so-called "Homogeneous-Resistance" in which the two colony variants differed for only one-fold MIC dilution.

The genomic Phylogeny (gPhyl), MLSTs and resistomes categorized the 9 *Ab* strains in 3 main clusters (cluster-I: gPhyl lineages-I, ST-OX 1839, COL Full-Resistance, Resistome-I, harbouring diverse non-synonymous *pmr*B SNPs; cluster-II: gPhyl lineages-II, ST-OX 1816 or ST-OX 218, COL Heterogeneous-Resistance, Resistome-II/III, carriers or not *pmr*B SNPs; cluster-III: gPhyl lineages-III, ST-OX 1808, COL Adaptive/Heterogeneous/Homogeneous-Resistance, Resistome-IV, *pmr*B SNP carriers and non-carriers).

No *Ab* strain harbouring *lpx*D/A/C SNPs were found.

Discussion and Conclusions

Our investigation, for the first time, find out the diversity of COL-resistance profiles among clinical COL^R *A. baumannii* defining the 4 different COL-R phenotypes and outline some genomic traits related to specific phylogenetic, MLST, resistome and *pmr*B-SNP clusters.

15 - Non-antibiotic biocides to restore the levofloxacin efficacy against multidrug-resistant Helicobacter pylori strains

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Introduction. Multidrug-resistance in *Helicobacter pylori* strongly stimulates the search for new strategies improving eradication rate. Novel approaches for tackling the antimicrobial-resistance phenomenon involve non-antibiotic biocides, so-called Antibiotic Resistance Breakers-ARBs, expressing antibacterial/anti-virulence activities restoring the efficacy of conventional drugs. This study analysed two ARBs, *Pistacia vera* L. oleoresin-ORS and Bovine Lactoferrin-BLF, for their antimicrobial, anti-virulence action and synergistic effect when combined with Levofloxacin-LVX against resistant *H. pylori* strains.

Materials and Mehods. ORS and BLF antimicrobial/anti-virulence effects against multidrug resistant *H. pylori* strains were analyzed *in vitro* by MIC/MBC determination, biofilm biomass quantification and bacterial motility on soft agar. The synergism with LVX was evaluated by checkerboard assay. *In vivo* studies were also performed using, for ORS, *Galleria mellonella* that is a recognized experimental model for *H. pylori* infection; and for BLF, a prospective therapeutic trial on two patient groups (one treated with esomeprazole/amoxicillin/LVX and the other with the same treatment plus BLF). Treatment outcome was determined by ^{13}C Urea Breath test.

Results. ORS and LVX MICs ranged, respectively, from 80 to 3120 mg/l and from 0.12 to 2.00mg/l. MBCs were similar to MICs. *In vitro*, ORS was able to synergize with LVX, restoring its effectiveness in LVX resistant strains. ORS, LVX and their synergistic combinations displayed a significant biofilm reduction. Moreover, ORS and LVX showed protective effect against *H. pylori* infection on *G. mellonella*. *In vitro,* BLF inhibited the growth of 50% of strains at 10mg/ml and expressed 50% bactericidal effect at 40mg/ml. The combination of BLF and LVX displayed a synergistic effect for all studied *H. pylori* strains, with the best MIC reduction of 32- and 16-fold for BLF and LVX, respectively. BLF at 1/4MIC reduced, significantly, the microbial motility for all strains. *In vivo,* 6 out of 24 recruited patients had treatment failure recorded with esomeprazole/amoxicillin/levofloxacin (75% success), and in the group with esomeprazole/amoxicillin/levofloxacin/bovine lactoferrin, 2 out of 53 patients recruited had failure recorded (96.07% success), with a therapeutic gain of 21%.

Discussion and Conclusion. Overall, our findings, from *in vitro* and *in vivo* studies, underline that the use of ORS and BLF, combined to LVX, could represent an effective and innovative strategy to tackle the antibiotic resistance and biofilm forming capability in *H. pylori*. ORS and BLF can be considered promising ARBs for restoring the LVX susceptibility in resistant *H. pylori* strains.

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16 - Isolation of a ST167 NDM-5-producing Escherichia coli strain from a kitten in Italy

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Introduction: *Klebsiella pneumoniae* carbapenemase and New Delhi Metallo-beta-lactamase (NDM)-Producing Enterobacteria (CPE) have been widely described in clinical and environmental samples. The number of CPE detected in livestock, wildlife and companion animals is increasing. Aim of the study was the genomic characterization of a NDM-5-producing *Escherichia coli* isolate collected from a cat of a private house.

Materials and Methods: An *E. coli* strain (167624/2), collected from a liver sample of a 4 months cat, died for parvovirus haemorrhagic enteritis, was identified and evaluated for antibiotic susceptibility with the semi-automated system AUTOSCAN4 (BeckmanCoulter). After DNA extraction with the DNeasy Blood and Tissue kit (Qiagen), and libraries preparation with the Nextera XT kit (Illumina), the Whole-Genome Sequencing (WGS) was performed on an Illumina MiSeq platform with a paired end run (2*250 bp). The reads quality was evaluated using FastQC, and assembled using Shovill. Detection of resistance and virulence genes was performed via Abricate; while the plasmid incompatibility groups (Inc) characterization using the on-line PlasmidFinder tools. For the phylogenetic analyses, the alignment and the coreSNP calling were performed using the software Purple on the 167624/2 and the 50 closest genomes, retrieved from PATRIC database. The coreSNPs alignments were used to infer phylogeny via RaxML with 100 bootstrap replicates.

Results: The 167624/2 strain showed a MDR profile, being resistant to all the antibiotics tested, but colistin, amikacin and fosfomycin. WGS analysis revealed a complex resistome for the strain, harbouring *bla*NDM-5, *bla*AmpC, *bla*AmpH, *aac(3)-Ila*, *aad*A2, *tet*R, *tet*A, *dfr*A12, *mph*A, *mdf*A, and *sul*1 determinants. The mutations in *par*C, *gyr*A and *par*E genes explained the fluoroquinolones resistance. Several virulence factors were also found: *fyu*A, *gad*, *iuc*C, *iut*A, *sit*A and *ter*C. The strain belonged to the ST167, clonal complex Cplx 10. The plasmid typing highlighted the presence of the IncI2(Delta), with a 100% identity, and the multireplicon IncFII, IncFIA and IncFIB (95%, 99,48% and 98,39% identity, respectively). The *E. coli* 167624/2 strain was phylogenetically closely related to other ST167 *E. coli* isolated from Italy and Switzerland, both of human and animal origin.

Discussion and Conclusions: The detection of ST167 NDM-5-producing *E. coli* from both clinical samples and companion animal sources has been rarely reported in Europe, including Italy. Due to the close contact between humans and pets, and the potential for cross-species transmission, the increase of carbapenemases found in companion animals represents a worrying trend.

21 - blaVIM harboring IncA plasmid from a clinical ST69 Escherichia coli strain in Italy

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Introduction: VIM (Verona Integron-encoded Metallo-beta-lactamase) is a member of the Metallo-beta-Lactamases (MBLs), able to hydrolyze beta-lactams antibiotics, including carbapenems, except for monobactams. To date, 69 different enzyme variants have been described, VIM-1 and VIM-4 being predominant in Europe. *The bla*VIM genes are reported in IncN, IncY, IncR and IncA plasmids. Recently, IncA group was recognized as alternative "reservoir" of carbapenemase genes in *Enterobacterales* family. Aim of the study was to characterize a VIM-producing IncA plasmid isolated from a clinical ST69 *Escherichia coli* strain from an Italian Long Term Care Facility (LTCF) inpatient.

Materials and Methods: On the 2nd of May 2018, a rectal swab was collected from a female patient, resident in the rehabilitation center "Giovanni Paolo II", Milan. Species identification and antimicrobial susceptibility testing were performed by Vitek-2 System (BioMérieux) and confirmed by AutoScan4 System (Beckman Coulter); interpretation was done according to EUCAST 2020. Phenotypic synergy test revealed that the *E. coli* strain produced a MBL-type enzyme. PCR and sequencing were used for carbapenemase identification. The transferability of the above determinant was verified by conjugation experiments. Whole-Genome Sequencing (WGS) of the strain was performed on extracted DNA using Sequel I platform. Genome assembly was executed using "Microbial Assembly". The resistance genes content and the plasmid typing were accomplished using the on-line tools ResFinder and PlasmidFinder databases from the Center for Genomic Epidemiology. The genome annotation was done by NCBI Prokaryotic Genome Annotation Pipeline (PGAP).

Results*:* The strain showed a Multi-Drug Resistance (MDR) profile, being susceptible only to amikacin, colistin, fosfomycin, meropenem, imipenem, ciprofloxacin. PCR and Sequencing assessed the presence of a *bla*VIM-1 gene variant. Conjugation assay confirmed the transferability of the *bla*VIM-1 gene. WGS revealed the presence of three contigs: the chromosome (4,962,700 bp), an IncA plasmid, p550_IncA_VIM_1 (162,608 bp), and an IncB/O/K/Z plasmid, p550_IncB_O_K_Z (100,306 bp). The p550_IncA_VIM_1 harbored *aac(6')-Ib3*, *aadA1*, *aph(3'')-Ib*, *aph(3')-XV*, *aph(6)-Id*, *bla*SHV-12, *bla*VIM-1, *mdf(A)*, *mph(A)*, *catB2*, *aac(6')-Ib-cr*, *qnrS1*, *sul1*, *sul2*, and *dfrA14* genes. The p550_IncB_O_K_Z plasmid resulted free of antibiotic resistance genes.

Discussion and Conclusions*:* We report the first detection of a VIM-1-harboring IncA plasmid in a ST69 *E. coli* strain in Italy. This work highlights the important role of IncA plasmids in disseminating the *bla*VIM-1 gene in Italian area

23 - A novel Beta-Defensin analogue is active against multidrug resistant bacterial strains

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Introduction: Today, infections associated with multidrug-resistant (MDR) bacteria still represent a great challenge to treat due to limited therapeutic options, thus requiring alternative antimicrobial strategies. The research to develop more sophisticated systems to effectively treat MDR bacteria is essential and represents one of the main challenges of the 21st century. Herein, we describe the major antibacterial features of a new molecule deriving from the human Beta-defensin 3 (hBD3).

Materials and Methods: The antimicrobial activity of the new peptide was evaluated against *P. aeruginosa* ATCC27853, *E. coli* ATCC13762, and *S. aureus* ATCC6538P and against MDR clinical isolates of Methicillin-Resistant *S. aureus* (MRSA), *E. coli* extended-spectrum Beta-lactamase (ESBL), *P. aeruginosa*, and *A. baumannii* complex. In particular, the MIC and the MBC of the peptide, both in the oxidized and reduced form, and the ability to influence biofilm formation and maturation and finally the bactericidal activity of the peptide by Time killing assays were evaluated.

Results: In this study, we have extended the previous characterization of a short structure within hBD3, and the new evidences confirmed that the new peptide displayed very interesting antimicrobial features. We have found that the molecule counteracts Gram-positive and Gram-negative reference strains and MDR bacterial strains. In particular, exhibited specific activity against *S. aureus* ATCC and *P. aeruginosa* ATCC strains, grown both in planktonic that in sessile form during the biofilm formation and maturation. Interestingly, the MIC and MBC values, obtained with both the oxidized and reduced form of the peptide, were very low against both reference bacterial strains and multi-resistant clinical isolates. Furthermore, time killing assays were performed to allow the assessment of bactericidal or bacteriostatic activity, as well as for the prediction of the pharmacodynamic profile of the novel peptide. The assays showed that peptide has very fast killing rates against all *S. aureus*, *E. coli* and *P. aeruginosa* tested strains at concentrations equal to MIC and 2xMIC, respectively. While the effect was bacteriostatic against *A. baumannii* with dose-dependent activity resulting in the highest percentage of bacterial growth inhibition at 4xMIC concentration.

Discussion and Conclusions: Overall, these data suggest that the antibacterial efficiency of the new analogue is comparable with the full-length hBD3 peptide and lead us to consider this molecule as a new and effective weapon against resistant multi-drug bacterial infections. The next step will be the evaluation of its potential in *in vivo* experiments also through the delivery by nanovector devices.

35 - Patterns of medically important antimicrobials resistance in Enterococcus spp. isolates from wild and domestic ruminants, Italy

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Introduction. Antimicrobial resistance (AMR) represents an important threat in terms of environmental contamination, public and animal health. There is limited information concerning the levels of AMR in wildlife, especially with regard to the antibiotic classes involved, the potential sharing of the habitat with the domestic animals and the level of environmental contamination caused by the human activities. In this study, the AMR patterns against selected medically important antibiotics were evaluated in commensal fecal bacteria from wild and domestic ungulates living in restricted areas of the Majella National Park, Abruzzi region, Italy.

Material and Methods. During October and November 2019, 35 fecal pools were collected from wild (red deer, chamois) and domestic (cattle, sheep and goats) ruminants by means environmental sampling. *Enterococcus* spp. isolates were obtained by a preliminary non-selective incubation of the samples in buffered peptone water, followed by a streaking on Slanetz-Bartley agar. The species identification of selected colonies and the antimicrobial susceptibility test for quinupristin/dalfopristin, vancomycin and linezolid molecules were carried out by Vitek 2 system (Biomerieux). Finally, the relative AMR genes were detected by multiplex, end-point, primers specific, PCR protocols.

Results. A total of 15 *E. gallinarum*, 12 *E. faecium*, 11 *E. faecalis*, 6 *E. hirae* and 4 *E. casseliflavus* were isolated. Among them, 18 out of 48 isolates were resistant or intermediate to at least one antibiotic and 7/48 isolates were multidrug resistant. Resistance to quinupristin/dalfopristin, linezolid and vancomycin was observed in 18 (37,5%), 8 (16,6%) and 7 (15,5%) isolates, respectively. For each class under study, the resistance genes were amplified in 11/18, 7/8 and 4/7 resistant isolates.

Discussion and Conclusions. Despite the resistant *Enterococcus* isolates were identified in both wild and domestic ungulates, the AMR patterns obtained in this study showed some differences in relation to the areas and the populations sampled. The phenotype resistance to linezolid and vancomycin was observed only in domestic and wild animals that sharing the grazing land, in comparison to the wild ungulates living in more isolated areas of the Park. In these territories the human activities are restricted, suggesting a minor risk of resistant bacteria spreading in the environment by the livestock. The results obtained in this study may provide relevant information about the potential spreading of multidrug resistant bacteria in the environment and they suggest the importance of a One Health, multidisciplinary approach in order to tackle the AMR challenge.

38 - Comparative genome analysis of two Streptococcus pneumoniae serotype 19A isolates: search for new non-PBP beta-lactam resistance determinants

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Introduction: *Streptococcus pneumoniae*, the pneumococcus, is a clinically relevant human respiratory pathogen responsible for more than a million deaths per year worldwide. Beta-lactam antibiotics have been employed to treat infections caused by *S. pneumoniae* for decades. However, penicillin-resistant *S. pneumoniae* strains, frequently expressing multiple antibiotic resistance phenotypes, have increased dramatically since the 1980s and pose serious problems in the treatment of infections. The Hungary19A-6 clone, a multiple antibiotic-resistant strain with unusually high-levels of beta-lactam resistance was prevalent in Hungary during the 1990s. This clone spread also in the Czech Republic and Slovakia but not significantly to other areas. In this work, we studied the resistance determinants in two serotype 19A strains from Hungary, strains Hu15 and Hu17. Both isolates are members of the same clonal complex, ST226, a single-locus variant of the representative strain HUN663 of the Hungary19A-6 clone. Hu17 exhibits high-level penicillin resistance, whereas Hu15 is penicillin sensitive. This unique situation was used to study the development of penicillin resistance and to understand the contribution of genes that might have played a role in conferring this highly resistant phenotype.

Material and Methods: In this study, the whole genome sequences of two *S. pneumoniae* clinical isolates from Hungary were compared using different bioinformatic tools.

Results: The mosaic structure of the genes encoding the penicillin-binding proteins (PBPs) from the resistant strain Hu17 and their influence in cefotaxime resistant in two different isogenic backgrounds were recently reported. In the present study, we focused on the analysis of the genomic regions flanking the *pbp* genes and estimated the size of recombinational replacements. Furthermore, single nucleotide polymorphisms (SNPs) were detected using the BIGSdb Genome Comparator program. We found that SNPs were dispersed across the genome, but they accumulated more in at least 6 large regions. In total, 62 loci contained SNPs. In 36 genes the presence of SNPs resulted also in changes at the protein level. SNPs were localized primarily in genes known to be directly involved in beta-lactam resistance, such as those encoding PBPs, but were also found in other genes involved in cell wall metabolism and cell division.

Discussion and conclusion: Taking together, these results support the notion that beta-lactam resistance in the pneumococcus is a complex event that involve modifications other than just PBPs and suggest that a specific genetic background is required to fully express resistance.

46 - New Resveratrol derivatives as promising strategy against Helicobacter pylori resistance

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Introduction. The increasing phenomenon of multidrug-resistance in *Helicobacter pylori* underlines the stringent need of novel strategies to improve the eradication rate. Nowadays, there is a great attention in alternative treatments based on combined synergistic effect between antibiotics and non-antibiotic compounds resulting in a potentiated effect. Over the last years, Resveratrol (RSV) aroused great attention due to its biological and antimicrobial activities, although *in vivo* application is limited for its poor bioavailability. In this study, we evaluated the antibacterial and anti-virulence effects of RSV and new synthetized RSV-phenol derivatives, alone and combined with Levofloxacin (LVX) used in *H. pylori* therapy, in areas where Clarithromycin resistance is major to 15%, against resistant clinical isolates, in *in vitro* and *in vivo* studies.

Materials and Methods. The RSV (lead compound) and RSV-phenol derivatives antibacterial activities were determined by MIC/MBC evaluation and the synergism with LVX through the checkerboard tests. The anti-virulence action was assessed by the motility inhibition, biofilm biomass reduction and anti-quorum sensing effect. The toxic and protective effects of RSV and RSV-phenol derivatives were also performed *in vivo* using the *Galleria mellonella* model that is a recognized experimental model for *H. pylori* infection.

Results. Among RSV-phenol derivatives, some compounds possessed higher antibacterial activity than RSV with a MIC reduction of 8-fold in respect to the lead compound, showing MIC values ranged from 25-6.25 mg/l. These compounds were also able to synergize with LVX reducing the LVX MIC values under the breakpoint. Moreover, a more interesting anti-virulence action of the detected RSV-phenol derivatives in respect to RSV was observed. The tested compounds displayed no toxic effect, showing a protective effect against *H. pylori* infection in *G. mellonella.*

Discussion and Conclusions. Overall, our data underline that the new synthesized RSV-phenol derivatives could be considered a potential strategy for innovative therapeutic schemes to tackle the *H. pylori* antibiotic resistance.

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48 - Antifungal activity of oleolites of Helichrysum and Hypericum against Candida spp. Multi drug - resistant

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Introduction Already known in ancient times, the oleolites, also called oil tinctures, are extracts of medicinal and aromatic plants obtained through the solvent action of action of a suitable vegetable fat oil. Oleolites are used in various fields such as cosmetics and phytotherapy mainly for topical use.

Materials and Methods In the extraction, extra virgin olive oil was used to ensure a good preservation of the oil. Both of the *Helichrysum* (*Helichrysum microphyllum Cambess. subsp. tyrrhenicum Bacch. Brullo & Giusso*) and the *Hypericum* (*Hypericum perforatum l. var. angustifolium*) was used the fresh drug, especially the flowers. The oleolites of Hypericum and Helichrysum were tested toward 15 strains of Multidrug-Resistant *Candida* isolated from several infection sites by patients of the U.S. of Dermatology of Sassari, thus determining the value of the minimum fungicidal concentration through the broth microdilution method. The isolated microorganisms included: three *C. albicans*, three *C. parapsilosis*, three *C. tropicalis*, three *C. glabrata*, three *C. krusei*, all.

Results Data analysis showed that *Helichrysum* oleolito had a higher fungicidal activity than *Hypericum* and Fluconazole.

Discussion and conclusion The increasing incidence of fungal infections and antifungal resistance has prompted the search for novel and effective antifungal drugs and alternative agents. Also, use of natural antifungal products could prevent development of resistance on antifungal drugs. The aim of this study was to find an alternative for antifugal drugs currently used in the treatment of fungal infections. In conclusion we can affirm from the obtained preliminary data, that the oleolito of *Helichrysum* could integrate standard antifungal drugs in the treatment of dermatological fungal infection.

56 - MOLECULAR ANALYSIS IN CARBAPENEM SUSCEPTIBLE AND CARBAPENEM RESISTANCE K. PNEUMONIAE STRAINS ISOLATES IN SICILY

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Introduction:
Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and mortality. The "classic" non-virulent strain of *K. pneumoniae,* producing extended-spectrum beta-lactamases (ESBLs), is associated with nosocomial infections. Hypervirulent *K. pneumoniae* strains are associated with invasive infections in previously healthy adult people, and most of them exhibit antimicrobial susceptibility. The role of virulent strains of *K. pneumoniae* (including hv-KP) in neonatal infections is unknown.
The aim of our study was to revenue of the distribution of *K. pneumoniae* in our geographic area and assessing the extent to which virulence determinants were carried by CR-Kp (carbapenem resistance *K. pneumoniae*) and CS-Kp (carbapenem susceptible *K. pneumoniae*).

Methods: 50 K. pneumoniae strains, 25 CR-Kp and 25 CS-Kp, collected from march 2015 to march 2017 were characterized for antibiotic susceptibility and fully sequenced by next generation sequencing (NGS) for the in silico analysis of resistome, virulome, multi-locus sequence typing (MLST) and core single nucleotide polymorphism (SNP) genotypes.

Results: By MLST in silico analysis we found that 52% of isolates of CR-Kp belonged to CC258, followed by ST395 (12%), ST307 (12%), ST392 (8%), ST348 (8%), ST405 (4%) and ST101 (4%). The in silico β-lactamase investigation of the CR-Kp group showed that the most detected gene was blaSHV (100%), followed by blaTEM (92%), blaKPC (88%), blaOXA (88%) and blaCTX-M (32%). The virulome analysis detected mrk operon in all studied isolates, and wzi-2 was found in three CR-Kp isolates (12%). Additionally, the circulation of virulence genes encoding for the yersiniabactin system, its receptor fyuA and the aerobactin system did not indication significant distribution differences between CR-Kp and CS-Kp.

Discussion and Conclusions: Our data suggest that the epidemiological frame in the Palermo area (Sicily, Italy) is present and new MDR clones are emerging. However, our analysis, which included the comparison of the virulence degree of CS-Kp and CR-Kp isolates, has unpredictably discovered that the latter are finding highly-virulent determinants and the co-presence of more resistance genes.

57 - Molecular characterization of carbapenem-resistance K. pneumoniae strains in Palermo-Sicily

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Introduction *Klebsiella pneumoniae* is a Gram-negative that cause opportunistic infections in hospitalised or immunocompromised patients. The antimicrobial resistance (AMR) due to horizontal gene transfer (HGT) is aided by plasmids and mobile genetic elements. Particularly, carbapenem-resistant *K. pneumoniae* (CRKP) strains have emerged as one of the ultimate challenges for public health because of their extended antibiotic resistance and ability to rapidly disseminate in the hospital. The spread of CRKP is mostly linked to the expansion of successful high-risk clones producing carbapenemases of various types and the main are KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo-β-lattamase), OXA-48 (carbapenem-hydrolysing oxacillinase-48), VIM (Verona integron-encoded metallo-β-lattamase) and IMP (Imipenemase type enzimes), with a paradigmatic example represented by the clonal group 258 (CG258), that is found in Europe and North America. In Italy, there is a surveillance of system with the aim of report all cases of bloodstream infection due to CRKP. In this study we report the analysis of 107 CRKPs from bloodstream infection of hospitalized patients of the Policlinico P. Giaccone of Palermo.

Materials and Methods From February 2017 until now, clinical isolates have collected from blood with antimicrobial MICs ≥ 8 μg/ml for meropenem and/or imipenem-resistance according to EUCAST clinical breakpoints and they are submitted to a rapid molecular typing. Detection of ST-258/512 clones were detected three genes that are *pilv-l*, *is-66* and *prp* by a multiplex PCR assay. While, the main carbapenem resistance genes that were analyzed are bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48} by a PCR assay.

Results The study showed that 26/107 CRKP isolates belonged to the clonal complex 258 (CC-258) with prevalence of 24%. Instead, as regards the main genes involved in carbapenem resistance, the data showed 67/107 bla_{KPC} (63%), 1/107 bla_{VIM} (0,9%), 1/107 bla_{NDM} (0,9%), 11/107 bla_{OXA-48} (10%), 0/107 bla_{IMP} and 5/107 bla_{KPC} + bla_{OXA-48} (5%). In addition, our study show that 18 of 26 CRKP isolates belonged to CC-258 and are positive a bla_{KPC}.

Discussion and Conclusion The high prevalence of bla_{KPC} suggests an increase of the epidemiology of CRKP population in the spread of this carbapenemase resistance gene. Furthermore, our study shows the high association between CC-258 and bla_{KPC}. Our findings support the need to keep a surveillance system to study the diffusion of carbapenemase resistance genes in our population.

60 - Antiviral Effect of Hornstedtia bella Škorničk essential oil from the whole plant, leaves, and rhizomes against vaccinia virus (VV).

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Introduction: In the prevention of epidemic and pandemic emerging and neglected viral infections, natural products are an important source of lead compounds. *Hornstedtia bella* Škorničkis is a rhizomatous herb growing in the forest of central Vietnam. Until now, no uses were reported, and we describe for the first time antiviral activity against a member of the Poxviridae family, Vaccinia virus (VV), closely related to variola virus, the causative agent of smallpox.

Materials and Methods: Hb EO was evaluated in *cell-based* assay for cytotoxicity, antiviral activity against a broad spectrum of viruses responsible for important human diseases and, its potential mode of action was characterized by virucidal studies followed by time-of-addition assay. Furthermore, the safety profile of Hb EO has analyzed trough the Transepithelial Electrical Resistance (TEER) experiment and, a combination assay with Mycophenolic Acid (MA) was performed.

Results: Hb EO was able to strongly reduce the viral VV titer in *in vitro* assay at not cytotoxic concentration. EO is not endowed of direct virucidal activity and, in a pre-treatment assay did not exert any inhibitory activity. Inhibition was instead observed when the Hb EO was added during the infection period. Its inhibitory effect is then exerted at an early step of the viral cycle. Hb EO did not affect the TEER and a combination treatment with Hb EO and MA, used as a reference compound, determines both, an improvement of antiviral activity and protection of monolayers from VV infection.

Discussion and Conclusions: In our studies, Hb EO was shown to be active against the Vaccinia virus inhibiting a step of its replicative cycle that occurs during the early phase of viral infection. The Hb EO safety profile shows that it did not affect the TEER and then the integrity of monolayer until 48 h post-treatment. Combining compounds with additive or synergistic antiviral effects is an established approach to enhance antiviral potency, reduce potential toxicity and damaging effects as well as to minimize the induction of potential drug resistance. The *in vitro* efficacy of Hb EO combined with MA increased the inhibitory effect of MA in a synergic way. These findings are encouraging and additional research is ongoing to investigate whether Hb EO can exert antiviral properties as a result of the complex interactions between their constituents or being associated with their main components.

64 - In vitro resistance and evolution of resistance to Tavaborole in Trichophyton rubrum

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Introduction. Onychomycosis is a nail fungal infection mostly caused by dermatophytes, particularly *Trichophyton rubrum*. The disease is often intractable for both the difficulty to reach effective drug levels at the site of infection and potential acquisition of resistance, leading to frequent relapses after therapy cessation. In addition, many currently available antifungals have been shown to induce cross-resistance to other drugs that share a similar mechanism of action. Tavaborole (Tvb) is the first member of a new class of boron-containing antifungals, developed for the topical treatment of onychomycosis caused by dermatophytes. Tvb targets the cytoplasmic leucyl-transfer ribonucleic acid synthetase, thus inhibiting fungal protein synthesis. In this study, we evaluated the emergence and evolution of resistance against Tvb in *T. rubrum*, which was chosen as model organism for clinically relevant dermatophytes.

Material and Methods. Four *T. rubrum* strains were used. Minimal inhibitory concentrations (MICs) were determined by the agar dilution assay. To evaluate the frequency of spontaneous mutants resistant to Tvb, *T. rubrum* strains were grown on Sabouraud Dextrose Agar (SDA) plates containing the minimal inhibitory drug concentration. To analyze the *in vitro* evolution of resistance to Tvb, strains were subcultured 10 times on SDA plates containing 0.5-fold the MIC. After the 5th and the 10th transfer, conidia were seeded on SDA plates containing 2-fold the MIC. To evaluate the spectrum of resistance of the mutants to other drugs (hydroxypyridone, azole, and morpholine antifungals), MICs were determined by the broth microdilution method.

Results. Spontaneous *T. rubrum* mutants resistant to Tvb were isolated with a frequency of 10^{-8} and showed a 2- to 4-fold increase in the MIC values of Tvb compared to the parental strains. In the presence of sub-inhibitory drug concentrations for 10 transfers, Tvb resistance frequency increased to 10^{-7} and 10^{-6} after the 5th and the 10th transfer, respectively. The level of Tvb resistance exhibited by the induced mutants was not different from that of the spontaneous mutants, suggesting that growth in the presence of sub-inhibitory Tvb concentrations does not cause variations in the level of resistance. No cross-resistance to others antifungal agents was found.

Discussion and Conclusions. Overall, this study indicates that spontaneous *T. rubrum* mutants resistant to Tvb can be isolated, although at low frequency, and that sub-inhibitory drug concentrations facilitate the emergence of resistant strains. The different mechanism of action of Tvb compared to other antifungals can explain the absence of cross-resistance development.

66 - Antifungal properties of selected essential oils and pure compounds on emerging Candida non-albicans species and uncommon pathogenic yeasts.

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Introduction. *Candida* spp. are the most important cause of opportunistic mycoses worldwide. Although more than 100 species of Candida have been described, the most challenging infections are caused by *C.albicans*. However, other *Candida* species and other rare yeasts are emerging as key opportunistic pathogens. Currently, the emergence of antifungal resistance is reported worldwide and is still an unresolved problem. Existing antifungal drugs have challenges to cope with the evolving nature of drug-resistant fungal pathogens. These issues have stimulated the search for new therapeutic alternatives, including essential oils (EOs) that are now well recognized for their remarkable biological activities including an antimicrobial role. Hence, the aim of this study was to investigate the *in vitro* antifungal activity of nine selected EOs and some main components against *Candida* non-*albicans* species, and other uncommon pathogenic yeasts clinical strains.

Materials and Methods. Commercial EOs of *Foeniculum vulgare*(fennel), *Lavandula vera* (lavender), *Melissa officinalis*(lemon balm), *Pinus sylvestris*(pine), *Salvia officinalis*(sage), *Thymus vulgaris*(thyme red), *Eugenia caryophyllata*(clove), *Origanum vulgare*(oregano), *Pelargonium asperum*(geranium)and EO main components (α-pinene, carvacrol, citronellal, eugenol, ɣ-terpinene, linalool, linalyl acetate, terpinen-4-ol and thymol) has been screened for antifungal activity against 47 different clinical *Candida* non-*albicans*(*C.krusei, C.parapsilosis, C.valida, C.lusitaniae, C.norvegensis*)and uncommon pathogenic yeasts (*Pichia etchellsii/carsonii, Kloechera japonica, Saccharomyces cerevisiae, Sporobolomyces salmonicolor*) clinical strains. Fluconazole (FLC) and voriconazole (VRC) were used as positive controls.We evaluated the Minimum Inhibitory Concentration(MIC) and the Minimum Fungicidal Concentration(MFC)of EOs, EO main components and drugs according to the CLSI M27-A3 with some modifications.The final concentrations ranged from 0.0019-1% v/v for EOs and components.

Results. Pine and lemon balm EOs showed the highest antifungal activity against non-*albicans Candida*, with MIC range of 0.03-0.12% (v/v).Other most active EOs were clove and geranium EOs (MIC range 0.06-0.25%v/v).Between compounds, α-pinene demonstrated the greatest inhibitory effect (MIC range 0.002-0.016% v/v).As regard uncommon pathogenic yeasts, oregano, pine, thyme red, α-pinene, carvacrol, eugenol and thymol showed the highest activity *in vitro.*

Discussion and Conclusions. These data showed a promising potential application of EOs as natural adjuvant for management of infections by emerging *Candida* non-*albicans* species and uncommon pathogenic yeasts. EOs and their components studied encourage adequately controlled and randomized clinical investigations.

81 - Increasing isolation of OXA-carbapenemase variants in Escherichia coli and Klebsiella pneumoniae strains not genetically related.

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Introduction. OXA-48-type carbapenem-hydrolyzing class D beta-lactamases are widely distributed among *Enterobacteriaceae*, with significant geographical differences. In Italy these enzyme are reported rarely.We notice an increase of isolates that they could be suspected for production of OXA-carbapenemases, in the last months in the Verona Hospitals and we investigated the OXA enzymes and genetic relationship of these strains.

Materials and methods. Four *E.coli* strains and 4 *K. pneumoniae* strains collected from purulent specimens at the Verona Hospitals and were investigated for carbapenemase production by Carba-NP and NG-Test® CARBA 5 phenotypic tests. Antibiotic susceptibility testing was performed by broth microdilution method. Ceftazidime/avibactam susceptibility was assessed through E-test. The presence of OXA-48 like enzymes was detected by PCR using the following designed primers: FW-TCGATTATCGGAATGCCTGC and Rev- GAGCACTTCTTTTGTGATGGC that gave a product of 735 bp. PCR products were purified using the Qiagen kit and sequenced (Eurofins Genomics, Germany). PCR Based Replycon Typing (PBRT 2.0 kit, Diatheva) was used for plasmid identification and typing. For the *E. coli* strains was performed also a PCR for the phylogenetic group classification. MLST was perform in order to assess the strains sequence type (Pasteur scheme).
Results. Broth microdilution method tests showed that the MICs for carbapenems were lower of breakpoint, except for two *K. pneumoniae* strains that were resistant and co-harboured also a KPC enzyme. At the NG-Test® CARBA 5 the strains resulted all positive for OXA-48 like enzymes. The CARBA-NP test was not able instead of detecting the presence of OXA-carbapenemases. All the strains were resistant to third generation cephalosporins, ceftazidime/avibactam showed no resistances. The presence of the bla_{OXA} genes was assessed through PCR and was confirmed in all the strains, amplicons were sequenced. OXA sequence analysis revealed OXA-505, OXA181 and OXA244. At the PBRT analysis *K. penumoniae* strains showed the same plasmid profile, while *E. coli* strains differed among them. A common plasmid found in these strains is the incL/N.
Both phylogenetic profile and MLST of the *E. coli* strains showed that strains are not genetically related. B1 group, A/C group and B2 group were identified. In the same way ST86, ST303 were identified in the MLST experiment.

Discussion. We register an increase isolation of OXA-carbapenemases in a hospital that is endemic for KPC enzyme. Note of worthy is the variability of the OXA enzymes variants. The strains harbouring OXA-carbapenemae are both *E. coli* and *K. pneumoniae* and also between the same species strains are not genetically related. This represent a worrying public health treath.

89 - RESISTANCE TO CARBAPENEME IN GRAM NEGATIVE BACTERIA: THE SPREAD OF blaNDM CARBAPENEMASE

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Introduction:
In recent years, carbapenem-resistant Gram-negative infections have been associated with an increase in morbidity and mortality and prolonged hospitalization which translates into a significant burden on healthcare system. In particular, the main carbapenem-resistant strains represented by *Klebsiella pneumoniae*, *Acinetobacter baumannii* and less commonly by *E.coli* and *Pseudomonas aeruginosa*, constitute a serious emerging problem. In order to limit the spread of carbapenem-resistant microorganisms, was conducted a surveillance in a period between June 2019 and July 2020. The unit of Intensive Therapy Unit (ICU) medical and surgery unit were involved in the study.

Materials and methods:
The colonization by carbapenem-resistant Gram-negative was evaluated by phenotypic methods. While the GeneXpert CARBA-R test was used to evaluate the genes correleted to the resistance. Resistant isolates were identified by MALDI-TOF.

Results:
Our results show that at hospitalization 12,82% (110/858) of the patients were colonized by Gram-carbapenem resistant. Particulary 72,73% (80/110) of the strains were positive for *bla*$_{KPC}$, while 2,73% (3/110) and 0,91% (1/110) were positive for *bla*$_{OXA-48}$ and *bla*$_{NDM}$ respectively. In addition, some strains were positive for two different genes: 4,55% (5/110) for *bla*$_{KPC}$/*bla*$_{OXA-48}$, 0,91% (1/110) for *bla*$_{KPC}$/*bla*$_{NDM}$ and 1,82% (2/110) for *bla*$_{KPC}$/*bla*$_{VIM}$. However, 16,35% of the strains resistent phenotypically to carbapenems did not showed any of genes analized. When the hospedalizzation was prolongated for more than one week the monitoring did not detected higer percentage of resistance, in fact 73% (73/100) of the strains were positive for *bla*$_{KPC}$, while 1% (1/100), 3% (3/100), *2%* (2/100), 4% (4/100) and 1% (1/100*)* respectively showed positivity for *bla*$_{NDM}$, *bla*$_{OXA-48}$, *bla*$_{KPC}$/*bla*$_{OXA-48}$, *bla*$_{KPC}$/*bla*$_{VIM}$ and *bla*$_{KPC}$/*bla*$_{VIM}$/ *bla*$_{NDM}$. Finally, 10% (10/100) resulted positive for *bla*$_{KPC}$/*bla*$_{NDM}$.

Discussion and Conclusion:
Spread of carbapenemase-producing Gram negative has been increasingly reported worldwide. Given the limited therapeutic options available, the accurate and timely detection of NDM -producing carbapenem-resistant Gram-negative is vital in order to control their spread. An international effort is needed to control the spread of these multiresistant pathogens. With limited treatment options, scientists need to develop new antibiotics and novel molecules to treat NDM-positive bacterial infections. There is an urgent need for infection control and continued global monitoring of isolates that harbor the NDM enzyme, as evidenced by recent outbreaks. Our results are in according to the international reports dimostrating that also in our geographic area the misure for control of the spread of resistant strains are not deferible.

101 - Synergistic activity of ceftobiprole against clinical Enterococcus faecalis isolates

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Introduction: Enterococci are intrinsically resistant to many commonly used antimicrobial agents, and may harbor different degrees of beta-lactam resistance. *Enterococcus faecalis* strains penicillin and ampicillin resistant are rare and have been associated with increasing production or aminoacidic substitutions of low-affinity PBP4. Moreover, penicillin-resistance among *E. faecalis* clinical isolates have been recently reported in many countries. This phenomenon is of a big concern, as synergistic combination with cell wall-active agents is frequently used in treatment of enterococcal infections. Ceftobiprole is an advanced cephalosporin that binds to multiple PBPs, leading to an *in vitro* antibacterial and bactericidal activity. We investigated the *in vitro* antibacterial and bactericidal activity of ceftobiprole and its synergistic activity combined with other beta-lactams against clinical *E. faecalis* isolates belonging to diverse antibiotic-resistance classes.

Materials and Methods: 40 clinical *E. faecalis* isolates belonging from bloodstream infections (BSIs) were analysed for their antibiotic susceptibilities and synergy testing was performed by gradient-cross method. The combination analysed were: ceftobiprole *plus* ampicillin, imipenem and piperacillin/tazobactam. Bactericidal activity of ceftobiprole and comparators was performed by Time-kill curves against representative isolates possessing diverse phenotypes of resistance to beta-lactams, and belonging to diverse antibiotic-resistance classes.

Results: Ceftobiprole $MIC_{50/90}$ were 2/8 mg/L. There was good agreement between gradient test and broth microdilution methods. 5 penicillin-resistant strains were detected, showing reduced susceptibility to ceftobiprole. Imipenem, ampicillin and piperacillin-tazobactam represented efficient combinations (81-85% synergistic/additive effect). In particular, *E. faecalis* showed largely synergistic effects in combination with imipenem (41%) and ampicillin (36%). Time-kill curve analysis demonstrated the potent ceftobiprole bactericidal activity, higher than that of the comparator drugs.

Discussion and Conclusions: Ceftobiprole exhibited a potent *in vitro* antibacterial and bactericidal activity against clinical *E.faecalis* strains, and a good synergistic activity in combination with all β-lactams tested, also against vancomycin-resistant and β-lactamase producing strains. The gradient-cross method provided a valuable alternative test to estimate synergism between cell wall-active agents, useful for a rapid evaluation of the efficacy of the combination therapy.

110 - Phenotypic and molecular characterization of an NDM-1-producing Acinetobacter baumannii of clinical origin

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INTRODUCTION. *Acinetobacter baumannii (Ab)* is a Gram-negative nosocomial pathogen, which frequently shows acquired multidrug resistance (MDR) to different antibiotic classes including beta-lactams, fluroquinolones, and aminoglycosides. Acquired class D carbapenemases production (OXA-23-like, OXA-24-like and OXA-58-like) and the over-expression of OXA–51-like driven by IS*Aba1* insertions, are the most common mechanisms of carbapenem-resistance. Moreover, NDM-producing *Ab* have been recently reported worldwide. Here we report the phenotypic and genotypic characterization of the first case of an NDM-1-producing *Ab* (AOUC-FI-11) detected in Italy from the bronchoalveolar lavage of an inpatient with pneumonia, reporting a travel history to Niger, Africa.

MATERIALS AND METHODS. The antimicrobial susceptibility test was performed by reference broth microdilution according to ISO 20776-1: 2019. Results were interpreted according to EUCAST v.10.0 clinical breakpoints (for cefiderocol, non-species related PK/PD breakpoints were used). The isolate was subjected to Whole-Genome Sequencing (WGS) by MiSeq (Illumina® San Diego, CA, USA) and MinION platforms (Oxford Nanopore Technologies, UK), and *de novo* hybrid assemblies were generated with Unicycler v. 0.4.6. *In silico* detection of resistance genes and the international clonal lineage (IC) was performed by specific webtools. Electro-transformation experiments were carried out using *A. baumannii* ATCC17978 as recipient host. Transformants were selected on Mueller-Hinton agar supplemented with 32 mg/L ceftazidime and screened for bla_{NDM} gene using Real-Time PCR.

RESULTS. AOUC-FI-11 was resistant to meropenem, gentamicin, cefiderocol and ciprofloxacin, but susceptible to amikacin (MIC = ≤4 mg/L) and colistin (MIC = 1 mg/L). WGS analysis showed that $bla_{\text{NDM-1}}$ was harbored on transposon Tn*125*, carried by a 66266 bp plasmid (pFI-AOUC-11) which also carried aminoglycosides (*aac(3)-IId*) and phenicol (*floR*) resistance genes. In silico MLST analysis showed that the isolate belonged to IC8, ST447. The pFI-AOUC-11 plasmid could be transferred to *Ab* ATCC17978. Transformant showed increased MIC values for carbapenems, cefiderocol and gentamicin, compared to the empty recipient.

DISCUSSION AND CONCLUSIONS. To our best knowledge, this is the first report of a clinical isolate of NDM-1-producing *Ab* in Italy, even if these isolates have been increasingly observed in Asia, Africa, South America and Europe, including France, Germany, Serbia and Slovenia. The isolation of an NDM-1-producing *Ab* in Italy, a country showing an endemic prevalence of carbapenem-resistant *Ab,* is alarming and its presence may be undetected without specific molecular characterization of the carbapenem-resistance mechanism.

111 - Repurposing drugs for treating multidrug-resistant lung infections by Pseudomonas aeruginosa in cystic fibrosis patients

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Introduction Cystic fibrosis (CF) patients are predisposed to chronic lung infection, mainly caused by *Pseudomonas aeruginosa*, which ultimately leads to pulmonary failure and death. The infection cannot be eradicated despite of regimens of nebulized Tobramycin (TOB), due to the multidrug resistance of *P. aeruginosa* and its ability to form inherently antibiotic-resistant biofilms. The "drug repositioning" approach – that is the finding of novel therapeutic indications for existing drugs – is a promising tool to search for antimicrobial discovery. In this study, 9 drugs were tested for their activity *in vitro* against selected *P. aeruginosa* isolates from CF patients.

Materials and Methods. Six *P. aeruginosa* isolates from CF patients were selected because strong biofilm producers (as assessed in microtiter plates by crystal violet assay) and multi-drug resistant (MDR) (i.e., an isolate resistant to at least three antibiotic classes among aminoglycosides, antipseudomonal penicillins, cephalosporins, carbapenems and fluoroquinolones). The susceptibility of *P. aeruginosa* isolates was assessed *in vitro* towards of TOB and 9 existing drugs: ribavirin (antiviral), toremifene (nonsteroidal antioestrogen), oxyclozanide (anthelmintic), meloxicam (nonsteroidal anti-inflammatory drug), apramycin (veterinary aminoglycoside), 5-fluorouracil (antineoplastic), actinomycin D (antineoplastic), furosemide (diuretic) and ciclopirox (antifungal). The minimal inhibitory and bactericidal concentrations (MIC, MBC) were measured using microbroth dilution method.

Results. Ribavirin, oxyclozanide, meloxicam and furosemide (MIC, MBC > 1024 µg/ml), and toremifene (MIC, MBC > 330 µg/ml) showed no antibacterial activity. Actinomycin D (MIC, MBC: 133 - >266 µg/ml), ciclopirox (MIC: 128-512 µg/ml; MBC > 1024 µg/ml) and 5-fluorouracil (MIC: 128 - >1024 µg/ml; MBC: 1024 - >1024 µg/ml) exhibited poor activity, although isolate-dependent. Apramycin was the most active drug, being active against all isolates with MIC values ranging from 8 to 64 µg/ml, whereas MBC ranged from 32 to 256 µg/ml. Furthermore, MBC/MIC ratio values (range: 1 – 4) indicated bactericidal mechanism of action. TOB MIC values ranged from 0.5 to >64 µg/ml; the activity of apramycin is not affected by tobramycin resistance.

Discussion and Conclusions. Apramycin is a unique member of the nebramycins and is used to treat Gram-negative infections in veterinary medicine. Our results showed it is active against MDR *P. aeruginosa* isolates, including those resistant to tobramycin. Further studies are needed to evaluate the applicability of apramycin to be used as a therapeutic antibiotic against *P. aeruginosa* lung infections in CF patients. In this regard, anti-biofilm assay and synergy studies with TOB are ongoing.

119 - SARS-CoV2 infection and multi-drug resistant bacteria superinfection in patients with mechanical assisted ventilation

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Introduction. We are facing the SARS-CoV-2 pandemic. At the moment no studies have specifically assessed COVID-19-associated superinfections and antimicrobial drug resistance. The aim of this work was to investigate the onset of bacterial infection regardless the time of Covid-19 infection in bronchoaspirate (BA) samples of patients with initial diagnosis of Covid-19 and need of assisted ventilation, and bacteria involved.

Material and Methods. 25 patients with Covid-19 infection and with assisted ventilation were evaluated also for bacterial superinfection. SARS-Cov-2 nucleic acid were extracted from BA by Seegene Nimbus and the target amplification was performed by Allplex 2019-nCoV assay kit (Seegene). BAs specimens were treated with dithiothreitol 1%, at 1X, 30 min at room temperature. 20 µl of treated samples were streaked out on medium plates and incubate at 37°C over-night. All isolated strains were identified by VITEK MS MALDI-tof (BioMérieux, France) system and antimicrobial susceptibility was determined using agar diffusion test.
Carbapenemase type was investigated by the NG-Test Carba 5 (Biotech).

Results All patients were hospitalized and treated at the beginning for Covid-19 infection and in all of them SARS-CoV-2 virus genomic sequences were detected and amplification usually start at early cicles. SARS-CoV-2 presence in the BA have been checked and monitored also in the subsequent to the adimission sample.
Only 3 patients out of 25 showed a negative or not significative culture for the time that they were followed. Twentytwo out of 25 showed at least one bacteria specie or even more.
The most frequently isolated bacterial species was *P. aeruginosa*, 17 patients out of 22 with positive cultures followed by *Klebsiella pneumoniae* in 8 patients out of 22.
Twelve strains out of 17 the *P. aeruginosa* strains were carbapenem resistant and were isolated with high load. Six patients showed a *K. pneumoniae* KPC producer. Except that in a patient that we isolated *S. pneumoniae*, a typical agent of community acquired pneumoniae, all other microorganims registered belong to the etiological agents of hospital acquired pneumoniae.
Note of worthy is that viral load have been decreased in all patients and bacteria took place of virus.

Discussion and Conclusions. 88% of the patients with Covid-19 in the intensive care unit developed a bacterial superinfection. These data suggest that bacterial infection may play an important role in the evolution of Covid-19. The most isolated microrganism was the carbapenem resistant *P. aeruginosa* followed by *K. pneumoniae* KPC producer, so most of the case register a MDR resistant microorganisms. Usually superinfection appear when the virus showed a lower viral load and then patients resulted negative for SARS-CoV-2 virus.

135 - Tedizolid-rifampicin combination prevents rifampicin-resistance on in vitro model of Staphylococcus aureus mature biofilm

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Introduction: *Staphylococcus aureus* infections associated with implanted medical devices are difficult to treat and require long-lasting antibiotic therapies, especially when device removal is not possible or easy such as in the case of joint prostheses. Biofilm formation is a major cause of treatment failure and infection recurrence. This study aimed to test, for the first time, the *in vitro* combination of tedizolid plus rifampicin on methicillin-sensitive and methicillin-resistant *S. aureus* mature biofilm. Clinical MRSA isolates from patients with biofilm-related bloodstream infections were included in this study in order to obtain preliminary results about the effect of rifampicin-tedizolid combination.

Materials and Methods: Minimal inhibitory concentrations of tedizolid, daptomycin, and rifampicin for two *S. aureus* reference strains (MSSA ATCC 6538 and MRSA ATCC 43300) and clinical isolates were determined. Mature *S. aureus* biofilms of the strains were treated with one or a combination of antibiotics at their MIC or multiples of the MIC. Biofilm biomass and the onset of rifampicin resistance were evaluated by crystal violet staining method and CFU count, respectively.

Results: The combination of tedizolid with rifampicin significantly disaggregated pre-formed biofilm of both reference strains at clinically meaningful concentrations. Notably, the onset of rifampicin resistance was completely prevented when biofilms were treated with tedizolid and rifampicin combination. Moreover these effects were similar to those obtained with daptomycin plus rifampicin, a well-known and widely used combination. Preliminary results on MRSA clinical isolates mirrored the efficacy of this combination in reducing biofilm biomass and preventing rifampicin resistance onset.

Discussion and Conclusions: Our results on *S. aureus* biofilm, together with previously available data, suggest a complementary activity of this combination. Indeed, rifampicin is known to be able to disaggregate *S. aureus* biofilm, while tedizolid has a good antimicrobial activity against planktonic bacteria and it has been demonstrated that inhibits *S. aureus* biofilm formation. So, it is easy to speculate that rifampicin and tedizolid combination has biofilm-disrupting ability and that could also prevent dissemination and subsequent biofilm regrowth from biofilm-detached cells; this is a usually encountered complication in biofilm-related infections leading to treatment failure and infections recurrences. Further *in vivo* studies are needed to confirm the validity of this promising therapeutic option that can be useful against biofilm-associated *S. aureus* infections.

3 IMMUNITA MICROBIOTICA E VACCINI

39 - Molecular characterization of S. pneumoniae causing invasive infections in Liguria (2017-2019)

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INTRODUCTION

Streptococcus pneumoniae is one of the main causes of invasive pneumococcal diseases (IPD), such as bacteraemia and meningitis in all age groups. Since 2010 the 13-valent pneumococcal conjugate vaccine (PVC13) was introduced for routine use in infants and in adults >64 years old people. Since serotype replacement is a common phenomenon in *S. pneumoniae*, serotype surveillance studies are mandatory to predict and monitor PVC13 coverage. In this study, the prevalence of serotypes causing meningitis and bacteraemia in Liguria between 2017 and 2019 was evaluated.

MATERIALS AND METHODS

116 cases of invasive *S. pneumoniae* infection (72 isolated by blood cultures and 44 by cerebrospinal liquid samples-CSF-) were reported in Liguria during 2017-2019. Pneumococcal strains were collected at the Microbiology Unit Policlinico San Martino, University of Genoa for further investigations. Identification results of all strains were confirmed by MALDI-TOF (Vitek MS bioMerièux, France) and by a species-specific PCR targeting the autolysin gene (lyt). Serotypes were determined by serotyping-specific Polymerase Chain Reaction (PCR).

RESULTS

Of the 116 strains, the most common serotypes in ranking order were: 3 (13.8%), 19 (7.75%), 6A and 9V (3,45% each one), 14 (2,6%),18C (1,7%) and 5 (0,86%). Seventy-seven (66.38%) pneumococcal strains belonged to non-PVC13 serotypes (55,4% and 65,9% blood and CSF isolates respectively).

DISCUSSION AND CONCLUSIONS

PVC13 has been introduced into national immunization programs since 2010, and international surveillance studies demonstrated that this vaccine had an additional impact, targeting the serotypes unique to PVC13, as well as continuing to protect against the PVC7 serotypes. Since the majority of our recently isolated strains belong to nonPVC13 serotypes, serotype replacement has occurred in our geographic area. In particular, in the last year, the strains belonging to nonPVC13 serotypes reached 85,7% of all isolates. This result shows that the PVC13 had a protective effect against the widespread invasive serotypes in the past, however, it may have played a decisive role in serotype replacement and spread of the other virulent strains that are not covered by the polysaccharide vaccines. Data shown in this study show that the burden of pneumococcal disease remains high and given the present scenario, IPD active surveillance is needed as well as continued research into novel vaccine development.

68 - Shelter from the cytokine storm: pitfalls and prospects in the development of SARS-CoV-2 vaccines for an elderly population

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Abstract
The SARS-CoV-2 pandemic urgently calls for the development of effective preventive tools currently not available. COVID-19 hits greatly the elder and more fragile fraction of the population boosting the evergreen issue of the vaccination of older people. The development of a vaccine against SARS-COV-2 tailored for the elderly population faces the challenge of the poor immune responsiveness of the older population due to immunosenescence, comorbidities and pharmacological treatments. Moreover, it is likely that the inflammaging phenotype associated with age could both influence vaccination efficacy and exacerbate the risk of COVID-19 related "cytokine storm syndrome", with an overlap between the factors which impact vaccination effectiveness and those that boost virulence and worsen the prognosis of SARS-COV-2 infection. The complex and still unclear immunopathological mechanisms of SARS-CoV-2 infection, together with the progressive age-related decline of immune responses, and the lack of clear correlates of protection, make the design of vaccination strategies for older people extremely challenging. In the ongoing effort in vaccine development, different SARS-CoV-2 vaccine platforms have been developed and tested in pre-clinical studies while only a small fraction of these are currently being tested in the older population. Recent advances in systems biology integrating clinical, immunologic, and omics data can help to identify stable and robust markers of vaccine response and move towards a better understanding of SARS-CoV-2 vaccine responses in the elderly.
Here, we reviewed the data on the impact of the elderly immune status in the response to vaccination and investigated the plans for inclusion of elderly subjects in clinical trials of candidate SARS-CoV-2 vaccines. We report that the major risk factors which are associated to and predict a poor prognosis of SARS-CoV-2 infection are in fact the same that hamper vaccination effectiveness in the elderly.

70 - Novel compounds to control Human herpesvirus 6 infection.

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Novel compounds to control Human herpesvirus 6 infection.
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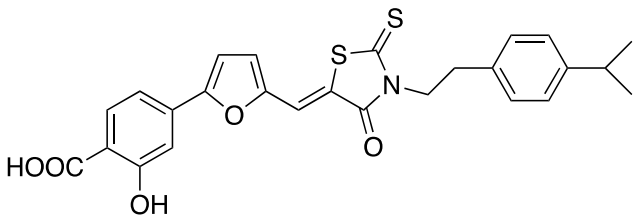
Introduction: An increased awareness of diseases associated with Human herpesvirus 6 (HHV6) infection and reactivation in both immunocompetent and immunocompromised patients has resulted in a growing interest in the evaluation of the best treatment options available for the clinical management of HHV6 disease. However, no compound has yet been approved exclusively for the treatment of HHV6. Thus, clinicians most often utilize the anti-cytomegalovirus (CMV) agents (ganciclovir, cidofovir and foscarnet) for the clinical treatment of HHV6, as in cases of HHV6 encephalitis. For this reason, the identification of anti-HHV6 compounds provides a valuable opportunity for developing efficient antiviral therapies.
Materials and Methods: We synthetized two different classes of molecules that present in one case the substituted rhodanine nucleus (1) and the second one the thiobarbituric moiety (2) (Figure 1) (Cagno V et al. 2018).



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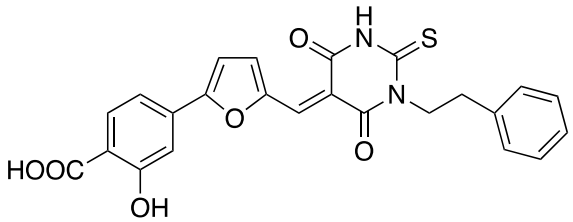

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Figure 1
The substituted furan was obtained via Suzuki coupling using the corresponding 5-formyl-furanyl-2-boronate and the 5-iodo-salicilic acid as a starting material for the synthesis of both compounds. The aldehyde obtained by this strategy has been used for a Knowenagel condensation with rhodamine derivative to produce compound 1 and thiobarbituric acid to obtain compound 2. Compounds 1 and 2 has been characterized by mono-dimensional and bi-dimensional NMR and by HPLC to confirm the structures and the purity grade. The two compounds were tested on HHV-6A and HHV-6B infected T cells. The levels of viral DNA, RNA and proteins were determined by Real Time PCR, RT-PCR and immunofluorescence.
Results: We report two compounds displaying an anti-HHV6A and HHV6B activity. The compounds inhibited both viral entry ($p=0.02$) and cell-to-cell transmission ($p=0.015$) in in vitro models of infection. These compounds are not cytotoxic and do not alter cell functions (protein expression, cell viability, mitochondrial activity). Due to their lipid oxidation ability, we hypothesize a mechanism on the viral envelope that affects the fluidity of the lipid bilayer, thus compromising the efficiency of virus-cell fusion and preventing viral entry.
Conclusions: These compounds present a selectivity for HHV6 envelop, without any effect on cell membrane. These results might be associated with the staticity of viral envelopes that are without any repair mechanism, in contrast with the biogenic membranes of the cells that are endowed with plenty of tools to repair membrane damage or alteration. We suggest a possible use of these new compounds to inhibit HHV6 life cycle and prevent disease progression.

96 - Characterization of a measles virus neutralizing monoclonal antibody targeting specific conformation of fusion protein

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Introduction. Measles virus (MV) remains one of the major causes of childhood morbidity and mortality worldwide despite extensive efforts towards its eradication through wide vaccination programs. The disease is generally self-limited but can lead to life-threatening complications related to the transient immune suppression and to the central nervous system (CNS) invasion. Among the antiviral treatments, the monoclonal antibodies (mAbs) become recently one of the most accepted therapeutics and a large number of neutralizing mAbs have been proposed against MV, mainly directed to the viral envelope glycoprotein H.

Materials and Methods. Here we have applied the results of fundamental research to identify and validate effective conformation-specific fusion (F) protein specific mAbs from a panel of hybridoma cell clones. These mAbs were tested for their neutralizing activity and their specificity *in vitro*.

Results. Plaque reduction assay showed that a mAbs clone endows a potent inhibitory effect on wild-type MV infection in target cells, and that it specifically detects the pre-fusion conformation of the MV F, both the wild-type and the CNS-adapted variant. In addition, we defined the sequence of the single-chain fragment variable (scFv) of the most effective mAb to design a small antibody fragment.

Discussion and Conclusions. Although the molecular basis remains incomplete, our results suggest that the neutralizing activity of the mAbs for MV infection represents a potential therapeutic strategy for prophylaxis in people at risk. The scFv is small enough to conceive its use also as potential treatment of severe CNS complications.

4 INTERAZIONI MICRORGANISMO-OSPITE

26 - Strigolactone Analogs Are Promising Antiviral Agents for the Treatment of Human Cytomegalovirus Infection

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INTRODUCTION: Human cytomegalovirus (HCMV) is a widespread lifelong pathogen whose infection usually doesn't produce overt manifestations in healthy individuals. On the other hand, in immunocompromised individuals, such as transplant recipients and AIDS patients, and if vertically transmitted to the fetus, HCMV can be associated with severe, even fatal, diseases. Although several drugs have been successfully employed against HCMV infection, their use is restricted because of toxicity, occurrence of serious side effects and viral resistance. Consequently, there is an urgent and unmet clinical need for less toxic, but highly effective, antiviral agents that can be safely administered against HCMV. Strigolactones (SLs) are a new emerging class of plant hormones with many and well-known functions in plant-related fields, while their roles on human cells and their potential applications in medicine are far from being fully exploited. In this context, the goal of this project is to define SLs antiviral activity during HCMV infection.

MATERIALS AND METHODS: We investigate the antiviral activity of a panel of SL analogs, called TH-EGO, EDOT-EGO, EGO-10, and GR24, in Human Foreskin Fibroblasts (HFFs) during HCMV infection via virus yield reduction assays. Focusing on TH-EGO and EDOT-EGO, we also employ attachment assays, entry assays and western blot analysis to clarify their effects during HCMV replication cycle. Furthermore, we investigate the capability of SL analogs to modulate cell-death pathways in HCMV infected cells using Annexin V analysis and *in-vitro* analysis of Caspase 3 activity. Finally, *in-silico* docking was used to predict possible viral molecular targets of SL analogs.

RESULTS: We demonstrate that TH-EGO and EDOT-EGO, and their derivatives TH-ABC and EDOT-ABC, significantly inhibit HCMV replication *in vitro*. Interestingly, SL analogs do not affect the first steps of HCMV infection - *i.e.*, attachment and entry - but dramatically impair late protein expression, including that of the HCMV tegument protein UL99 (pp28). Finally, we suggest that the SL-dependent induction of apoptosis in HCMV infected cells, during the late stages of infection, is a contributing mechanism to SL antiviral properties.

DISCUSSION AND CONCLUSIONS: These findings indicate that SL analogs TH-EGO and EDOT-EGO may be promising candidates for a new class of antiviral agents for the treatment of HCMV infections.

36 - Bioactive liposomes for the control of Mycobacterium abscessus infection

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Introduction. *Mycobacterium abscessus* (MA) is an opportunistic pathogen intrinsically resistant to many antibiotics, which is frequently linked to chronic pulmonary infections in immunocompromised patients. Cystic Fibrosis (CF) is a genetic disease characterized by Transmembrane conductance Regulator channel (CFTR) impaired functions, that is often associated to chronic infections, including those due to MA, causing high morbidity and mortality rates in CF patients. In this study, we have evaluated the efficacy of apoptotic body like liposomes loaded with phosphatidic acid (ABL/PA), phosphatydilinositol 3-phosphate (ABL/PI3P) or phosphatydilinositol 5-phosphate (ABL/PI5P), all bioactive lipids involved in the activation of phagocytosis machinery, both *in vitro* and *in vivo* models of MA infections.

Materials and Methods. Differentiated THP-1 cells (dTHP-1), used as a model of human macrophages, were treated or not with CFTR inhibitor, *in vitro* infected or not with MA, and finally stimulated with selected liposome formulations. ABL efficacy was evaluated in terms of i) bacterial uptake, ii) phagosome acidification, iii) ROS production and iv) intracellular mycobacterial killing. The efficacy of treatment with selected liposome formulations was also evaluated in *in vivo* model of chronic infection with MA, in terms of leukocyte recruitment and pulmonary bacterial burden.

Results. Results show that *in vitro* stimulation with ABL loaded with PA, PI3P or PI5P of human macrophages, treated or not with inhibitor of CFTR, promotes both MA internalization and intracellular killing. Moreover, dTHP-1 cells, infected or not with MA, show a significant ROS production and phagosome acidification following liposome stimulation. Finally, the aerosolic treatment with ABL carrying PA, PI3P or PI5P in the murine model of chronic MA infection induces a significant improvement in pulmonary mycobacterial clearance, which is associated with the reduction of leukocyte recruitment.

Discussion and Conclusions. Altogether, our results support the possibility of using bioactive liposomes as host-directed strategy that may represent an additional therapeutic tool for the control of MA infections, with the advantage to reduce the potentially tissue damaging inflammatory reactions observed during chronic pulmonary infections.

37 - Different modulatory effects of two different methicillin-resistant Staphylococcus aureus clones on MG-63 cells.

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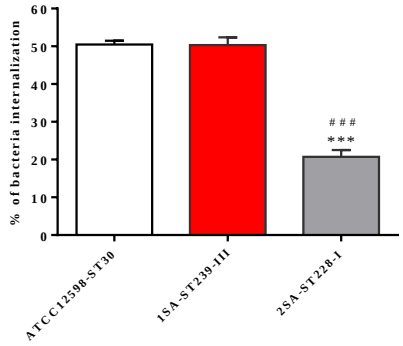
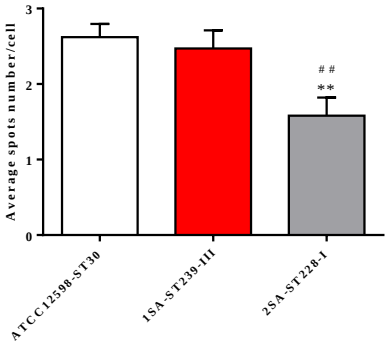
Introduction: It is well-known that *Staphylococcus aureus* is able to invade and persist within non-professional phagocytic cells such as osteoblasts. In the present study the toxicity of two different Methicillin-resistant *S. aureus* (MRSA) strains, 2SA-ST239-III and 10SA-ST228-I, and of the Methicillin-sensitive *S. aureus* (MSSA) ATCC12598-ST30 used as control, was tested in MG-63 osteoblast cells. We also determined their influence on the gene expression of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), transforming growth factor-β1 (TGF-β1), and metabolic marker glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as well as of nuclear factor E2-related factor 2 (Nrf2) and its downstream effector heme oxygenase 1 (HO-1). Materials/methods: The intracellular frequency of the three different bacterial strains was evaluated with Immaging Flow Cytometry at a multiplicity of infection (MOI) of 100:1. The evaluation of internalization and persistence on the viability of MG-63 cells was performed by MTT assay. Gene expression analysis was performed by quantitative Real-Time PCR, using human Quantitec primers. All the experiments were performed at 3 and 24 hours p.i. with bacterial strains. Results: Despite the already demonstrated similar abilities of internalization and persistence of ATCC12598-ST30 and 2SA-ST239-III strains in MG-63 under our experimental conditions, we demonstrated that the decreased cell viability due to the presence of bacteria inside cells is not proportionally related the number of cells. We demonstrated that 2SA-ST239-III was the only clone of *S. Aureus* able to significantly increase the gene expression of both pro-inflammatory cytokines IL-6 and TNF-α in MG-63 osteoblast cells at both time points; 2SA-ST239-III was also able to determine a strong up-regulation of TGF-β1 and GAPDH mRNAs 24 hours p.i.; neither the ATCC12598 nor the two MRSA induced oxidative stress phenomena in MG-63 cells (no changes in NADPH oxidase and inducible nitric oxide synthase gene expression), although a very different expression pattern towards Nrf2 and HO-1 activation was observed among the different clones.
Conclusions: Our results, can open a new way of considering therapies, going in the direction of an individualized therapeutic strategies that should take into account the difference existing between MSSA and MRSA as well as the distinctive features of the different clones. Not only, therefore, a different antibiotic approach but also a starting point for considering possible therapeutic strategies against staphylococcal damage such as the inhibition of TGF-β1 production or the selective inhibition of TGF-β1 signaling.

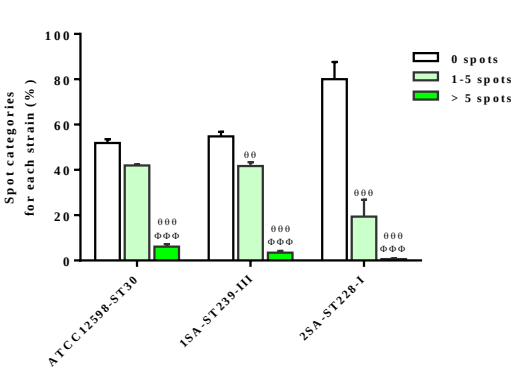
A


% of bacteria internalization
60
50
40
30
20
10
0
###
***
ATCC12598-ST30
1SA-ST239-III
2SA-ST228-1

B


Average spots number/cell
3
2
1
0
##
**
ATCC12598-ST30
1SA-ST239-III
2SA-ST228-1

C


Spot categories for each strain (%)
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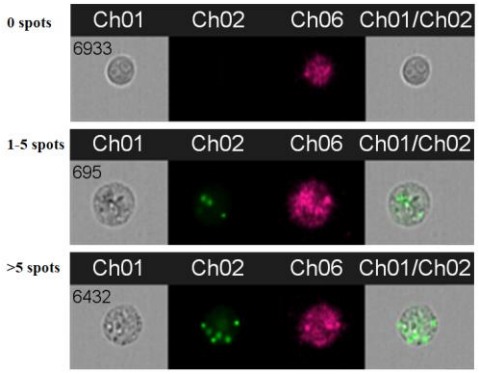
60
40
20
0
0 spots
1-5 spots
> 5 spots
θθθ
ΦΦΦ
θθ
ATCC12598-ST30
1SA-ST239-III
2SA-ST228-1


0 spots
Ch01
Ch02
Ch06
Ch01/Ch02
6933
1-5 spots
695
>5 spots
6432

40 - Canine Coronavirus (CCoV-II) infection provokes autophagy and impairs cellular iron metabolism

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Introduction - CCoV is a single positive-stranded RNA virus, belonging to Coronaviridae family. It mainly causes enteric disease in young puppies. Generally, in virus-cell host interaction, viruses takeover cellular machinery, inducing alteration in the regulation of numerous metabolic processes. For example, iron metabolism is essential, hence infection may be influenced by cellular iron status. Indeed, to efficiently replicate, several viruses require iron-replete host. Furthermore, it has been established that some viruses specifically infect cells through binding to transferrin receptor 1 (TfR-1), the main protein involved in cellular iron up-take. Iron metabolism has been proposed as a potential therapeutic target during infections. To date, the role of iron in coronaviruses infection has still to be elucidated. Here, we have thereby investigated the cellular response to CCoV infection, evaluating the iron homeostasis regulation in a canine fibrosarcoma cell line (A-72).

Materials and methods - A-72 cell line, bioscreen *in vitro*, cytomorphological and Western blot analysis.

Results - Following CCoV infection in A-72 cells, by bioscreen and morphological analysis, we detected signs of apoptosis, as previously observed, and autophagy. Apoptotic cell death was accompanied by a decrease of anti-apoptotic surviving protein. Autophagic alterations were due to increased levels of LC3-II and Beclin 1. The influence of CCoV infection on cellular iron metabolism was evaluated by investigating the expression of the main proteins involved in maintenance of iron homeostasis. We detected an up-regulation of TfR-1 expression associated to a down-regulation of ferritin, the most important iron storage protein. We further detected a reduction of superoxide dismutase (SOD) expression, which indicated an impairment of cellular antioxidant defence.

Discussion and Conclusions - CCoV, an important canine coronavirus, stimulated both apoptosis and autophagy. Furthermore, by enhancing iron up-take and reducing its sequestration in ferritin, a deregulation of iron metabolism occurs resulting in iron-dependent oxidative stress, as indicated by failure of cellular antioxidant mechanism. Overall, these preliminary results suggest a potential central role for iron metabolism during coronavirus infections.

41 - Citrullination and Herpes Simplex Virus type 1 infection: implications for neurodegenerative disorders.

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INTRODUCTION: Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that infects most humans, attaining 90% prevalence by the sixth decade of life. The disease appears as cold sores, typically seen on the lips or face, with primary infection usually during childhood. The virus remains latent in neuronal cell bodies and reactivates due to stress, illness and other unknown factors throughout an individual's life. In some cases, individuals can develop adverse reactions such as herpes simplex encephalitis (HSE). Moreover, recent evidence suggests the involvement of HSV-1 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 (PTM) catalyzed by peptidyl arginine deiminases (PAD) that convert peptidylarginine into peptidylcitrulline, whose dysregulation has been associated with Alzheimer's disease and others neurodegenerative disorders. Against this background, the goal of this project is to characterize the citrullination during infection with HSV-1 in different *in vitro* models. MATERIAL AND METHODS: We investigated the PADs expression profile in Human Foreskin Fibroblasts (HFF), African green monkey kidney cells (VERO) and Human Neuroblastoma Cell Line (SHSY-5) during HSV-1 infection using both Real Time quantitative PCR and Western Blot analysis. Furthermore, we determined the pattern of citrullination during infection using a citrulline-specific rhodamine phenylglyoxal (RhPG)-based probe.

RESULTS: We demonstrate that HSV-1 triggers PADs expression in all tested cell lines both at mRNA and protein levels. Interestingly, HSV-1 replication rate is strongly impaired in the presence of Cl-amidine and BB-Cl-amidine, two specific pan-PAD inhibitors, indicating that citrullination is required for HSV-1 replication. Interestingly, the overall citrullination profile obtained with the citrulline-specific probechanges consistently at different time points during infection.

DISCUSSION AND CONCLUSION: These findings could shed light on the role of HSV-1 in the pathogenesis of AD, providing new molecular mechanisms that could be exploited for advanced medical interventions.

44 - Phage-resistance mechanism in KPC-producing Klebsiella pneumoniae of the clonal group 258 clade II lineage

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Introduction. Antibiotic resistance is recognized by the World Health Organization as one of the most important threats for public health of the 21st century, due to a wide dissemination of multidrug-resistant (MDR) bacteria. Bacteriophage therapy is a promising alternative to conventional antibiotics, notwithstanding the need to characterize these viruses before their potential clinical use. A required major piece of information for the use of phages in therapy is the definition of the molecular targets exploited to infect bacteria. This also allows the rational design of phage cocktails, so to limit the onset of resistant mutants. In this work, the genetic basis of resistance to the lytic phage phiBO1E by its susceptible host *Klebsiella pneumoniae* (KPN) KKBO-1 has been studied. This strain is a member of the clade II of the clonal group (CG) 258, which is the main CG responsible of the worldwide diffusion of KPN producing the KPC-carbapenemase.

Materials and Methods. KKBO-1 mutants resistant to phiBO1E were obtained *in vitro* by exposure of KKBO-1 at high multiplicity of infection. Lysogeny was excluded by a PCR assay using primers targeting phage-specific genes. One mutant, designated BO-FR-1, was selected for subsequent experiments. Virulence of BO-FR-1 was evaluated in the *Galleria mellonella* infection model in comparison with the parental strain. Whole-genome sequencing (WGS) of BO-FR-1 was performed to explore potential genetic differences with KKBO-1.

Results. All randomly selected phage-resistant mutants (n=9) exhibited a rough phenotype and were not lysogenic. Infection with BO-FR-1 was significantly associated (p value< 0.0001) with lower mortality (*log* LD_{50} at 72 hours ≥8 CFU/larva) when compared to that of the parental strain (*log* LD_{50} at 72 hours of 7.171 ± 0.3465 CFU/larva). Analysis of WGS data showed that BO-FR-1 differed from KKBO-1 by a single nonsense mutation into the *wbaP* gene, which encodes a glycosyltransferase involved in the first step of the biosynthesis of the capsular polysaccharide (CPS).
Discussion and Conclusions. Results from this study suggest that the target used by this phage to infect KKBO-1 is a component of the CPS. Indeed, it is expected that the non-sense mutation observed in the *wbaP* gene leads to a lack of the production of the CPS by the phage resistant strain. Consistently, BO-FR-1 exhibited a rough phenotype and was less virulent than its parental strain in the *G. mellonella* infection model, providing further evidences about the major role of CPS for KPN virulence. Altogether, these results suggest that, in the context of the interplay among phage, bacterial pathogen and host, the emergence of bacterial resistance to the phage may be beneficial for the host.

47 - Co-presence of Streptococcus pyogenes and SARS-CoV-2 in the upper way tract of children; an important missing information?

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Introduction The peak of incidence of pediatric pharyngo-tonsillitis by group A streptococci (GAS, *Streptococcus pyogenes*) is during the winter-spring season. It is therefore likely to presume that, during the SARS-CoV-2 pandemic, the co-presence of *S. pyogenes* with SARS-CoV-2 could have taken place in the upper airways tracts of children. Unfortunately, no data are available to determine if and at what extent a such co-existence really happened.

Materials and Methods A retrospective analysis was performed on children, aged 0-14 years, by investigating records from the Clinical Microbiology Laboratory at University Hospital "Sant'Orsola-Malpighi" in Bologna to retrieve laboratory data on the SARS-Cov-2 test and how often it has been associated to GAS detection.

Results From February 20th to May 20th 2020, 1627 nasal-pharyngeal swabs for performing the SARS-CoV-2 test were collected from 1318 children. Overall, the positivity of the test was 8.1 %, corresponding to 105 children (8.0 %).
The screening for bacterial pathogens was requested in 4.8% of SARS-CoV-2 positive children and in 7.4% of SARS-CoV-2 negative children. In particular, GAS exposure (culture and/or antistreptolysin-O, ASO titers) has been investigated in 1.5% of SARS-CoV-2 positive children and in 4.3% of SARS-CoV-2 negative children (a total of 53 children).
Only one child had a throat swab positive for GAS; GAS exposure by high ASO titers (above 200 IU/ml) was confirmed in 35.9% children tested. All children but one were SARS-Cov-2 negative.
Even if individual clinical symptoms were not available, data analysis (extended until June 1th) indicated that 31 children were admitted in COVID Units. Only four children had a positive SARS-CoV-2 test. Noteworthy, ASO titers were investigated in twenty children and eight presented with high values indicating a recent/ongoing GAS infection was present. So, when investigated, a recent or ongoing GAS infection was concomitant in 40 % COVID-19 children.
The child positive for both SARS-CoV-2 and high ASO titers presented with a multisystemic inflammatory response attributable to the MIS-C syndrome, affecting some COVID-19 children.

Discussion and Conclusion During the pandemic emergency, swab testing focused on the identification of SARS-CoV-2 carriage in children mostly excluding the search of other possible pathogens. Nevertheless, our data indicated that the co-presence of GAS and SARS-CoV-2 could not have been a negligible entity.
We encourage the search for bacterial pathogens, in particular for *S. pyogenes* (cultural and ASO tests), along with SARS-CoV-2 test in children to collect evidence for a possible role of this common pediatric throat pathogen in influencing viral colonisation and modulating the infectious course in children.

55 - Structural analysis of Merkel Cell Polyomavirus (MCPyV) Viral Capsid Protein 1 (VP1) in HIV infected individuals.

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[8]IRCCS San Raffaele Pisana, Telematic University, Rome, Italy;
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Introduction: The surface of Merkel cell polyomavirus (MCPyV) virions is composed of pentameres of the major capsid protein, VP1. VP1 mediates attachment of the virus to cell surface, initiating infectious entry and delivery of the encapsidated viral genome to host cells. MCPyV plays a causal role in Merkel cell carcinoma (MCC), a highly lethal skin cancer. The events connecting MCPyV infection and cell transformation involve the integration of viral DNA into the host genome and the continued expression of the C-terminal truncated large T (LT) antigen. In addition to LT truncation, mutations in VP1 have also been supposed to contribute to the pathogenic properties of polyomaviruses. Given the limited information on this topic, the aim of this study was to investigate the genetic variability of MCPyV VP1 among an HIV+ population, in an attempt to clarify its effect on protein structural organization, membrane binding and antibody escape.

Materials and Methods: Thirty-four samples, simultaneously positive to MCPyV DNA in urine and plasma, from 17 HIV+ individuals, were subjected to VP1 amplification. Amplified fragments were sequenced and analyzed by using bioinformatics tools.

Results: The nucleotide sequence and the amino-acid translation of 27/34 VP1 displayed in urine and in plasma a wild type structure compared to the MCPyV prototype strain (MCC350). Seven/34 samples (3 urine and 4 plasma) showed a VP1 characterized by several nucleotide mutations such as transversions, deletions and insertions. The amino-acid analysis revealed, in 2/3 urine, a VP1 characterized by Ser28 to Tyr and Phe115 to Ile substitutions, located in the VP1 N-terminus and in the β-sandwich *core*, respectively. In one HIV+ individual, due to a frameshift mutation, the protein translation was not feasible, both in urine and in plasma. Regarding the amino-acid analysis of the remaining 3/4 plasma, the substitution Val69 to Asp and the Tyr79 deletion were observed in one sample within the first neutralizing epitope loop, whereas Ser251 was changed to Phe in the apical loop. For 2/4 plasma, stop codons did not allow VP1 translation.

Discussion and Conclusions: This is the first study in which MCPyV VP1, among HIV infected individuals, was analysed on the basis of the protein structure. The variations detected were unique and involved all the VP1 epitopes. The substitution in the neutralizing loop, may contribute to MCPyV immune escape. The mutation in the apical loop can lead to the selection of the viral variants with changed tropism. To address the consequences of the VP1 rearrangements on the MCPyV pathogenesis, *in vitro* studies are in progress in order understand if these amino-acids mutations have any structural or clinical implications.

61 - A case of intestinal Anisakiasis

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Introduction A case of a patient with acute abdomen caused by intestinal Anisakiasis, fish- trasmitted infection that results from ingestion of *Engraulis encrasicolus* contaminated by larvae of *Anisakis pegreffii,* a round worm from the order of Ascaridida.

Materials and Methods A 54-year old man, presented to the emergency room of the Policlinico Paolo Giaccone Hospital Palermo Sicily with sever abdominal pain, nausea and vomiting, without fever.The patient revealed that he had eaten marinated anchovies a few days earlier.

Discussion On the exploratory laparotomy an edematous and stenotic tract of 13 cm of jejunum was found, and a segmental resection was performed. Histologically, the resected jejunum showed a conspicuous and diffuse inflammatory infiltration, predominantly made up of eosinophil granulocytes, numerous mucosal erosions and the presence in the submucosa of sections of parasites which were identified as *Anisakis pegreffii* larvae. In addition, in collaboration with Buccheri La Ferla hospital, the serological examination was carried out for the detection of specific IgE towards anisakis, both to the patient and to the other diners who ate anchovies and who presented allergic symptoms.

Conclusion Globalization is responsable of the more and more frequent Anisakiasis disease worldwide, as increased mixture of different cultures and international traveling has allowed the spreading of risky feeding habits. Therefore anisakiasis must be taken into consideration in the differential diagnosis of intestinal obstruction in patients with a history of suspected consumption of raw or undercooked fish. Serological diagnosis by implementing both sensitive and specific methods is important for the diagnosis of gastro-allergic anisakiasis.

69 - The U24 protein of HHV-6A induces the expression of Alzheimer's disease risk factors of microglial cells.

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Introduction: In Alzheimer's Disease (AD) brains, pathological characteristics are observed: extracellular insoluble senile plaques formed by amyloid-β (Aβ) peptide, apoE (Lazar 2013) and intraneuronal neurofibrillary tangles (NFT) formed by tau protein (Kumar 2015). Recent findings suggest a possible implication of HHV-6A in AD, and we showed the ability of HHV-6A infection to induce the expression of apoE and to be involved in Aβ expression by microglial cells and cell activation.
Several evidences reported that a particular HHV-6A protein, U24, appears to be involved in the neurogenerative processes due to its high homology with MBP protein. Furthermore, U24 is able to induce tau hyperphosphorylation and Aβ expression through the activation of Fyn-kinase, a kinase involved in tau phosphorylation and Aβ induction, suggesting its role also in AD pathogenesis.
We evaluated the effect of HHV-6A infection, and particularly of U24 HHV-6A protein, on microglial cells expression of the common risk factor for AD development, apoE, Aβ and tau., and its involvement in Fyn-kinase activation and microglia migration.

Materials and Methods: We have infected microglial cells (HMC3, ATCC®CRL-3304), in monolayer and spheroid 3D model, with HHV-6A (strain U1102) cell-free virus inocula with 100 genome equivalents per 1 cell. We collected the cells 1, 3, 7 and 14 days post infection (d.p.i.). We also treated microglial cell with U24 protein. We analyzed viral DNA and RNA, apoE, Aβ (1-40, 1-42), tau and phospho-tau (Threonine 181), Fyn-kinase activation and cell migration by Real Time, immunofluorescence, immunoenzymatic assay and transwell migration assay.

Results: We observed a productive infection by HHV-6A that leads to increased apoE and Aβ 1-42 expression, and also of Fyn-kinase activation and tau/ptau percentage from 7 d.p.i. After treating microglial cells only with HHV-6A U24 protein, we observed the comparable results, also in term of migration induction.

Conclusions: Microglial cells are permissive to HHV-6A infection, that induces the expression of the common risk factor for AD development: apoE, Aβ and tau, together with the stimulation of microglia activation and migration. Interesting, we can observe the same induction treating microglial cell with the only HHV-6A U24 protein, with the involvement of Fyn-kinase.

71 - Sars-Cov-2 Spike 1 protein controls Natural killer cells activation via HLA-E/NKG2A pathway.

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Introduction: Natural killer cells are important in the control of viral infections. However, the role of NK cells during Sars-Cov-2 infection has previously not been identified. Peripheral blood NK cells from Sars-Cov and Sars-Cov-2 naïve subjects were evaluated for their activation, degranulation, interferon-gamma expression in the presence of Sars-Cov and Sars-Cov-2 spike proteins.

Materials and Methods: K562 and lung epithelial cells were transfected with spike proteins and co-cultured with primary NK cells from Sars-Cov-2 negative subjects. The analysis was performed by flow cytometry, immune-fluorescence, enzyme immunosorbent assay.

Results: Sars-Cov and Sars-Cov-2 spike proteins did not alter NK cell activation in K562 in vitro model. On the contrary, Sars-Cov-2 spike 1 protein (SP1) intracellular expression by lung epithelial cells resulted in NK cell reduced degranulation. Further experiments revealed a concomitant induction of HLA-E expression on the surface of lung epithelial cells and the recognition of a SP1-derived HLA-E-binding peptide. Simultaneously, there was the up-modulation of the inhibitory receptor NKG2A/CD94 on NK cells when SP1 is expressed in lung epithelial cells. We ruled out GATA3 transcription factor as responsible for HLA-E increased levels and HLA-E/NKG2A interaction as implicated in NK cells exhaustion.

Conclusions: We show for the first time that NK cells are affected by SP1 expression in lung epithelial cells via HLA-E/NKG2A interaction. The resulting NK cells exhaustion might contribute to immunopathogenesis in Sars-Cov-2 infection.

72 - Prevalence of airway-specific pathogens of Primary Ciliary Dyskinesia patients, afferent to PCD Reference Centre of Pisa, from 2014 to 2020.

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Introduction: Primary ciliary dyskinesia (PCD) is a rare genetically heterogeneous disease of mobile cilia with defective ciliary structure and/or function. In PCD patients cilia are immobile or have altered beat, and don't provide efficient mucociliary clearance: this results in bacterial colonization and eventually, in recurrent and chronic airway infections. As already known for Cystic Fibrosis, infection by *Pseudomonas aeruginosa* are correlated with worse lung function and clinical outcome of patients. However, few detailed information about infection causing pathogens are available, and there is a lack of microbiological data of PCD patients in Italy. The aim of the present study is to provide an overview about prevalence and susceptibility of the most common respiratory pathogens isolated from patients followed at the PCD Reference Centre of the University Hospital Pisa over the last 7 years.

Materials and Methods: A total of 994 respiratory specimens were collected from 2014 to 2020 from 139 PCD patients, with age ranging from 6 to 66 years. The clinical samples were analysed in the Microbiology and Virology Laboratories of the University Hospital of Pisa where bacterial and fungi identification and drug susceptibility test were performed. Clinical and microbiological data were analysed cross-sectionally and longitudinally.

Results: 358 out of 994 samples revealed non-pathogenic bacterial population. From the remaining 636 samples were isolated 31 different bacterial species: *Pseudomonas aeruginosa* was the most common bacterial pathogen (n = 344; 34,6%), followed by *Staphylococcus aureus* (n = 119; 12%), *Haemophilus influenzae* (n = 84; 8,45%) and *Streptococcus pneumoniae* (n = 64; 6,44%). Coinfection by *Pseudomonas aeruginosa* and other species were detected in 97 samples (9,76%). *Candida spp* and and *Aspergillus spp* were isolated in 269 and 81 respiratory specimens respectively. In the population as a whole, prevalence of *Pseudomonas aeruginosa* increase, while *Staphylococcus aureus* and *Haemophilus influenzae* decrease with age.

Discussion and Conclusions: The microbiological study of respiratory specimens of PCD patients showed that *P. aeruginosa* was the most abundant pathogen, followed by *S. aureus*. Some differences between the population were highlighted in terms of age of patients and bacterial prevalence. This study provides valuable information about the prevalence of the most common pathogens in PCD patients and could help in the characterization of PCD airway-specific flora, which is nowadays mostly unknown.

73 - Murine model of invasive enteric infection with Salmonella enterica serovar Typhimurium

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Introduction. Invasive Nontyphoidal *Salmonella* (iNTS) infection is a major cause of death in sub-Saharan Africa and currently no licensed vaccine against iNTS is available. Improved animal models that closely mimic human infection are needed to study *in vivo* iNTS pathogenesis as well as host responses and vaccine induced protection. Among iNTS, *Salmonella enterica* serovar Typhimurium is an invasive strain not host restricted to man, able to cause gastroenteritis as well as systemic disease in the mouse model, resembling infection with *S.* Typhi in humans. In the present study, a murine model of enteric infection with *Salmonella* Typhimurium was set up in susceptible C57BL/6 mouse strain. Animal survival, bacteremia, leukocyte counts and cytokine/chemokine levels in blood were investigated.

Materials and Methods. C57BL/6 mice were infected intragastrically (IG) with 10^7 or 10^8 colony forming units (CFU)/mouse of *Salmonella* Typhimurium ATCC strain 14028, an invasive strain. Survival of mice was monitored up to 10 days post infection, with a daily recording of body weight and clinical score. Bacterial counts were analysed daily in blood, and 5 days after infection in spleen, liver and mesenteric lymph nodes. Innate cell populations were characterized in blood by multiparametric flow cytometry, and cytokines/chemokines levels in serum were evaluated using Luminex immunoassay.

Results. With the higher dose of 10^8 CFU/mouse, was observed a survival of 80%, 35% and 0% six, seven and eight days post infection respectively. The inoculum of 10^7 CFU/mouse determined a survival of 80%, 50%, 18% and 0% three, five, seven and ten days post infection respectively, and was selected as optimal infection dose. A progressive weight loss and a simultaneous increase in the clinical score with the progression of the disease was observed. Bacterial loads were analysed five days post infection with 10^7 CFU/mouse, recovering about 10^5 CFU/ml of blood, 10^6 CFU/spleen and liver, and 10^5 CFU/mesenteric lymph nodes. The presence of *Salmonella* Typhimurium in blood was daily investigated and was recovered starting from day 4 after IG infection. The analysis of innate cell populations in blood of infected mice indicated a significant increase in the presence of neutrophils, monocytes and dendritic cells compared to naïve mice.

Discussion and Conclusions. In the present work, we have set up a murine model of enteric infection with invasive *Salmonella enterica* serovar Typhimurium, that mimics the natural oro-fecal route of infection. Bacterial dissemination, disease progression and cellular immune responses were characterised. Taken together, these data provide an important model to study host/pathogen interaction and protection conferred by *Salmonella* Typhimurium vaccines.

76 - ACE2-modulating compounds for the treatment of respiratory viral infections

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Introduction: ACE2, the SARS-CoV2 receptor, is a member of the renin-angiotensin system (RAS) that hydrolyzes Ang-II to form Ang (1-7) eliciting anti-inflammatory pathways and modulating intracellular redox state. Despite its role in viral entry, the protective role of ACE2 in the pathogenesis of respiratory lung infections, including SARS-CoV and influenza virus, was demonstrated in many *in vitro* and *in vivo* studies. Indeed, during respiratory viral infection the down-regulation of ACE2 has been related to the induction of Acute Respiratory Distress Syndrome (ARDS). This evidence suggests that the use of compounds that modulate ACE2 expression could decrease the severity of infection and ameliorate its outcomes. RAS interfering compounds such as Stilbenes has been also reported to impair the replication of several viruses, suggesting that ACE2 modulation could affect viral life-cycle. The aim of this study was to evaluate the effect of Resveratrol (RV) analogue compounds, such as Polydatin (RV glicoside form) and A5+ (Stilbene compounds and Sirtuin-1 activator), on ACE2 modulation and their efficacy against respiratory viral infections.

Materials and Methods: ACE2 expressing cell lines were treated with different concentrations of Polydatin and A5+ compounds (ranging from 5 to 30 μg/ml) for 24hrs, then the cytotoxicity of compounds was evaluated by MTT assay. No cytotoxic concentrations of Polydatin and A5+ were selected to treat cells infected with influenza A PR8/H1N1 virus. After 24hrs ACE2 protein expression was analyzed by Western blot, and viral titer was evaluated by Hemagglutination assay (HAU) and In Cell Western (ICW), through quantification of viral protein expression, nucleoprotein (NP) and hemagglutinin (HA), on infected cell monolayers.
Results: We found that both Polydatin and A5+ compounds (used at 20-30 μg/ml) increased by 30% the expression of ACE2 in mock-infected cells (controls) with respect to untreated controls. In addition, A5+, used at higher concentration (30 μg/ml) counteracted the PR8-induced decrease of ACE2, up-regulating the protein expression at the levels of mock-infected cells. Moreover, both compounds were able to significantly decrease hemagglutinating activity. A5+, at concentration ranging between 20-30 μg/ml inhibited by 50% viral infectivity measured in terms of NP and HA expression in infected cells.

Discussion and Conclusions: Overall data indicate that stilbene derivatives up-regulate ACE2 expression and inhibit influenza virus replication in host cells. Further studies are needed to clarify the mechanisms underlying the antiviral effect of ACE2 modulators and to explore their potential efficacy against respiratory viruses including SARS-CoV2.

77 - Emergence of equine-like G3P[8] strains in Italy since 2017

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Introduction
Rotavirus (RVA) is a major etiologic agent of gastroenteritis in human and animal babies worldwide. RVAs are classified in G and P-types on the basis of antigenic/genetic diversity of the capsid proteins VP7 and VP4, both eliciting neutralising antibodies. In addition, whole-genome classification, used to assign genotypes to each gene, allowed to classify the majority of human RVAs into Wa-like (G1/3/4/9/12-P[8]-I1-R1-M1-A1-N1-E1-H1) and DS-1-like (G2-P[4]-I2- R2-M2-A2-N2-E2-H2) constellations. Interspecies transmission and/or reassortment events can often occur driving RVA evolution. Recently, novel equine-like G3P[8] RVAs, with a DS1-backbone, emerged and spread worldwide. This study describes the genetic characterization of G3P[8] strains detected in Palermo from 2004 to 2019 in order to better understand their genetic evolution over the time.

Materials and Methods
Over 15 years of viral gastroenteritis surveillance, a total of 7044 stool samples, collected from children hospitalized at the "G. Di Cristina" Children Hospital of Palermo, were tested. All positive strains were G/P typed by hemi-nested PCR and five G3P[8] strains, representative of the study period, further investigated by full genome analyses according to the genotyping recommendations of the Rotavirus Classification Working Group.

Results
Over the study period, RVA accounted for 23.3% of gastroenteric infections and G3 genotype was detected in 8.9% of RVA-positive samples. Whole genome sequencing showed the presence of two population of G3P[8] strains. A Wa-like backbone (G3-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) was detected until 2017, while more recent strains exhibited a DS1-like backbone (G3-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2). Phylogenetic analyses of VP7 gene confirmed a high genetic correlation of the Italian G3P[8] DS1-like strains to equine-like RVAs emerged in several countries from 2013. Based on the VP4 phylogenetic tree, all Italian G3P[8] strains clustered together with an high percentage of nucleotide and amino acid identity (>95%). Comparison of VP7 and VP4 sequences to RotaTeq vaccine showed the presence of several aa substitutions in antigenic regions.

Discussion and Conclusions
G3P[8] equine-like RVAs emerged in Palermo in 2017 contributing to the seasonal epidemic in 2018 and 2019. Italian G3P[8] strains with a DS-1-like constellation were closely related to strains recently detected in several countries demonstrating their stable circulation in humans. Such novel G3P[8] strains represents a further demonstration of the evolutionary capability of RVAs. Therefore, RVA surveillance is important to detect promptly the emergence of new mutants and to ensure the effectiveness of vaccine programs.

79 - Multiple Herpes Simplex Virus-1 (HSV-1) Reactivations Induce Protein Oxidative Damage in Mouse Brain

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Introduction: Alzheimer disease (AD) is a neurodegenerative disorder characterized by brain accumulation of beta-amyloid plaques and neurofibrillary tangles, high levels of oxidative stress markers and neuroinflammation. Indeed, AD brains show evidence of reactive oxygen species (ROS)-mediated injury and increased levels of oxidative stress markers. Interestingly, HSV-1 has been reported linked to both AD and to oxidative stress conditions. Compelling evidence supports the role of herpes simplex virus-1 (HSV-1), a DNA virus able to establish a lifelong latent infection in sensory neurons with periodic reactivations, as one of the potential risk factors for AD. Recent studies, including ours, supported the hypothesis that multiple reactivations of the virus reaching the central nervous system (CNS) may predispose the brain to AD and shed light on the possible mechanisms underlying HSV-1 detrimental effect in the CNS. HSV-1 life cycle is reported to induce an intracellular redox imbalance in host cells, and *in vivo* evidence demonstrated that this occurs also in the brain. Here we investigated whether multiple virus reactivations induced oxidative stress in the mouse brain and affected the function of two proteins previously linked to AD, such as glucose-regulated protein 78 (GRP78) and collapsin response-mediated protein 2 (CRMP2) which are involved in the unfolded protein response (UPR) and in microtubule stabilization, respectively.

Materials and methods: BALB/c mice were infected with HSV-1 via snout abrasion and virus reactivation was periodically induced by thermal stress (TS). After 7 TSs, the levels of the oxidative stress marker 4-hydroxynonenal (HNE) was measured in brains of mice undergone multiple HSV-1 reactivations by dot-blot. A redox proteomic approach was used to identify those HNE-modified proteins mostly modulated by recurrent HSV-1 reactivations into the brain. The levels of GRP78 and CRMP2 expression and peroxidation were analysed by western blot, immunofluorescence and immunoprecipitation.

Results: Following multiple HSV-1 reactivations, mouse brains showed increased levels of oxidative stress hallmarks and 13 HNE-modified proteins whose levels were found significantly altered in the cortex of HSV-1-infected mice compared to controls. We found that recurrent HSV-1 infection inactivates the GRP78 function, thus affecting its capability to bind misfolded proteins and aberrantly activating UPR. On the contrary, the recurrent infection preserves the oxidative and phosphorylative status of CRMP2, likely preserving its function.

Conculsions: Overall, these data suggest that repeated HSV-1 reactivation into the brain may contribute to neurodegeneration also through oxidative damage.

87 - Redondovirus DNA in human respiratory samples

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Introduction: Redondovirus (ReDoV) is a recently discovered circular, Rep-encoding single-stranded DNA (CRESS-DNA) virus in humans. Its pathogenesis and clinical associations are still completely unknown.

Materials and Methods: The presence of ReDoV DNA was investigated in biological specimens of 543 Italian subjects by in-house developed PCR assays.

Results: The overall ReDoV prevalence was about 4% (23 of 543 samples). The virus was detected in 22 of 209 (11%) respiratory samples. One stool sample was also ReDoV positive. Viral DNA was not found in blood samples from immunocompetent and immunosuppressed subjects and cerebrospinal fluids from patients with neurological diseases. Genomic nucleotide differences were detected among the ReDoV isolates by sequencing a 582-nucleotide fragment of the capsid gene of the viral genome. Although we found no clear evidence that ReDoV was the direct cause of disease in the subjects studied, the most virus-positive patients had more severe respiratory diseases and no other common respiratory viruses and/or microbial agents.

Discussion and Conclusions: The results demonstrate that ReDoV circulates in Italian population, and that it's mainly present in the respiratory tract of infected people. Further investigations are needed to reveal possible clinical implications of this new CRESS-DNA virus in humans.

94 - Toll-like receptor 4-mediated inflammation triggered by extracellular IFI16 is enhanced by lipopolysaccharide binding

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Background: Since its discovery in the early 90s, a cornucopia of biological activities has been attributed to the IFI16 protein, including cell cycle regulation, tumor suppression, apoptosis, DNA damage signaling, virus sensing, and virus restriction. In addition, aberrant IFI16 expression and release in the extracellular space has been reported in a series of inflammatory conditions. The current hypothesis is that overexpression of the IFI16 protein occurs in tissue compartments where it is not physiologically expressed during inflammation. The ensuing release of the IFI16 protein into the extracellular space may allow it to behave like a damage-associated molecular pattern (DAMP) that signals through the Toll-like receptor 4 (TLR4) triggering inflammation by itself or through interaction with exogenous molecules, e.g. lipopolysaccharide (LPS).

Materials and Methods: Pull down assays and ELISA were used to characterize IFI16 binding activity to LPS. The human monocytic cell line THP-1 and the renal carcinoma cell line 786-O were used as target cells to define IFI16-induced proinflammatory activity. Co-immunoprecipitation (co-IP), surface plasmon resonance (SPR), and silencing experiments were used to define IFI16 signaling.

Results: We show that the IFI16 HINB domain binds to the lipid A moiety of either high or weak TLR4 agonist LPS variants. Treatment of THP-1 or 786-O cells with IFI16 led to increased production of proinflammatory cytokines, which was further enhanced when IFI16 was pre-complexed with sub-toxic doses of high TLR4 agonist LPS but not low agonists. Silencing of TLR4/MD-2 or MyD88 abolished cytokine production. These findings alongside with other in vitro binding experiments indicate that IFI16 interacts and signals through TLR4.

Discussion and conclusions: Collectively, our data provide compelling evidence that: *i)* IFI16 is a DAMP that triggers inflammation through the TLR4/MD2-MyD88 pathway; and *ii)* its activity is strongly enhanced upon binding to LPS variants regarded as full TLR4 activators. These data strengthen the notion that extracellular IFI16 functions as DAMP and point to new pathogenic mechanisms involving the crosstalk between IFI16 and subtoxic doses of LPS.

95 - SARS-CoV-2 infection in a bone marrow recipient: case report

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Introduction. Severe acute respiratory syndrome (SARS) is a potentially life-threatening, atypical pneumonia that results from infection with a novel virus, SARS-associated coronavirus (SARS-CoV-2). Limited information exists regarding the effects of this recently identified human coronavirus on transplant patients, and few case reports describe if SARS-CoV-2 infection is associated with an increased risk for complications and death in bone marrow transplantation. Here, we report a laboratory-confirmed case of SARS-CoV-2 infection in a 60 years old patient how had previously received bone marrow transplantation in Livorno hospital.

Materials and Methods. At the onset of symptoms related to coronavirus disease (COVID-19), three nasopharyngeal swabs (NPS) and a blood sample were collected and sent to the Virology Unit at Azienda Ospedaliero-Universitaria Pisana for molecular and serological tests. SARS-CoV-2 RNA detection was carried out in NPS by using two different real-time PCR assays, commercially available (*Roche* and *Seegen Inc*). The presence of SARS-CoV-2 IgG antibodies was investigated in serum samples by using the chemiluminescence immunoassay assay (*DiaSorin*). In addition to these analyses, a quantitative in-house neutralization assay was carried out to determine whether the neutralizing antibodies (NAbs) were present.

Results. The positivity to molecular test, and therefore the presence of SARS-CoV-2 in respiratory tract, persisted for three months until the appearance of NAbs at low titer. Noticeable, and perhaps explaining the long persistence of the virus, CLIA assay did not reveal the presence of IgG antibodies.

Discussion and Conclusion. Current absence of the virus possibly correlates with the presence of NAbs. This observation shows how the production of neutralizing antibodies may be fundamental for SARS-CoV-2 clearance and helps investigating of immunological aspects, also in strongly immunosuppressed patients.

114 - Evaluation of dexamethasone phosphate in the larvae infection model Galleria mellonella on the virulence of Escherichia coli and Staphylococcus aureus

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Introduction. *Galleria mellonella* is a well-accepted infection model used as an alternative to a mouse model to investigate the virulence of bacterial pathogens. Moreover, *G. mellonella* has been used as *in vivo* models in many bacterial pathogenicity studies, host-pathogen interaction and testing new antibiotic treatments. This alternative model has several advantages, such as fewer ethical concerns, low cost, precise bacterial inoculation and/or drug dosage, ability to incubate larvae at human body temperature, and ease of use for high performance screening of new *in vivo* treatments. The study aimed to assess whether immunosuppression with dexamethasone could be applied to the *G. mellonella in vivo* infection model through both *in vivo* virulence assays and gene expression studies.

Materials and Methods. Three different assays have been performed. A toxicity study with increasing concentrations of dexamethasone to assess the mortality of *G. mellonella* larvae. Subsequently, non-lethal doses of the glucocorticoid were co-administered with *E. coli* and *S. aureus* to assess its influence on virulence. Finally, the gene expression of Nf-kappa B and glutathione peroxidase of dexamethasone treated larvae at 24 and 48 hours was assessed.

Results. Larvae exposure to increasing doses of dexamethasone has demonstrated high tolerability of this insect to this glucocorticoid. Moreover, treatment of larvae with dexamethasone enhanced the lethality induced by infection with *E. coli* or *S. aureus* in a dose-dependent manner. As regards gene expression, after 48 hours dexamethasone altered NF-kappa B expression levels, while glutathione peroxidase expression remained unchanged.

Discussion and Conclusions. Larvae exposed to the drug become more susceptible to *E. coli* and *S. aureus* infections and die faster than their untreated equivalents. This increased lethality occurs due to the uncontrolled proliferation of bacteria within the infected larvae which can be at least partially attributed to the inhibitory effect of the drug confirmed by gene expression studies.

115 - Inhibition of SARS-CoV-2 infectivity by a peptidodendrimer designed on the viral spike protein

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Introduction. The Coronavirus disease 2019 (COVID-19) appeared in Wuhan City (China) in December 2019 and it spread worldwide with great speed. Everyday the number of deaths grows drastically, reaching 640000 deaths globally and over 14 million reported cases since the start of the outbreak. The etiological agent of COVID-19 was identified in a novel coronavirus denominated Severe acute respiratory syndrome-associated coronavirus 2 (SARS-CoV-2). It belongs to the large family of *Coronaviridae*, consisting of enveloped RNA viruses with a broad host range and the property of causing zoonotic diseases, such as SARS and Middle East respiratory syndrome (MERS). The infections lead to mild to severe respiratory illness and even death. It was widely reported that the surface spike (S) protein is essential for the coronavirus binding and entry of host cells. It is a class I fusion protein, characterized by two subunits: S1 binds to the human receptor angiotensin-converting enzyme 2 (hACE2), meanwhile the S2 subunit takes part in the viral and target cell membrane fusion. The S2 subunit contains several main elements, including a hydrophobic fusion peptide, a pair of heptad repeat (HR) helices and a pre-transmembrane domain. The S protein undergoes different conformational changes: the fusion peptide inserts into the target cell membrane and the two HRs rearrange to form a six-helices bundle (6-HB) leading to the fusion between the viral envelope and cellular membrane.

Materials and Methods. Starting from the entire aminoacidic sequence of the viral S protein (NCBI YP_009724390.1), a peptide designed on the C-terminal HR (H-GINASVVNIQKEIDRLNEVAKNLNESL-OH) was synthetized by solid-phase methodology and purified by reversed-phase high performance liquid chromatography. Then a solution of peptide was added to dendrimer, the mixture was concentrated and purified by size-exclusion chromatography. The anti-SARS-CoV-2 activity and the cytotoxicity of the peptidodendrimer were evaluated through viral plaque reduction and MTT assays, respectively.

Results. The peptidodendrimer was examinated for its ability to inhibit SARS-CoV-2 plaque formation on Vero cells, at peptide concentrations of ~30 μM. To validate that the observed antiviral activity was not a consequence of cellular cytotoxicity, a MTT assay was performed. Vero cells treated with ~30 μM of peptidodendrimer showed no difference in absorbance as compared to untreated cells.

Discussion and Conclusions. Peptides derived from the HR helices of the class I viral fusion proteins have been demonstrated to possess a strong antiviral activity, also against SARS and MERS viruses.

The addition of dendrimer allows the peptide to be targeted close to the spike C-terminal HR preventing the formation of the 6-HB and, as a result, the fusion virus-cell membranes. These findings allow for the identification of possible therapeutic peptides for the treatment of SARS-CoV-2 infections.

116 - Human-beta defensins-2 and -3 enhance the integrity of intestinal barrier ad protect the gut from Candida albicans infections.

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Introduction: the integrity of the intestinal barrier is maintained by a single layer of epithelial cells held together by apical protein complexes called "tight junction" (TJ). Alteration of the mucus layer and TJ causes an increase in intestinal permeability, followed by microbial translocation and systemic disorders. *Candida albicans*, in addition to the role of commensal, is an opportunistic pathogen responsible for disseminated candidiasis especially in immunocompromised subjects, where the damage of the intestinal mucosal barrier leading to dysbiosis. In this work we have created a line of intestinal epithelial cells capable of stably expressing the gene that encodes human beta defensin-2 (hBD-2) and 3 (hBD-3) to monitor the invasion of Candida in *vitro*.

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; *Evaluation of expression of tight-junction protein*: the mRNA and protein extracted from differentiated cells were respectively used for Real-Time PCR and Western Blot to evaluate the expression of Occludin, Zonulin-1 and Claudin-1. *Transepithelial Electrical Resistanceevaluation:* TEER measurement was evaluated after 21 days of culture.*Invasiveness assay*: the transfected and untransfected cells were infected with *C.albicans* at a ratio 1: 10 cell/yeast for 2 hours at 37°C. After this time the keratinocytes were treated with nystatin at a mycocidal concentration for 4 h at 37 °C,then were lysed with0.1% Triton-X100, serially diluted and incubated on SABat 30 °C to identify the viable intracellular yeast. *Apoptosis evaluation*: the transfected and untransfected cells were infected with *C.albicans*at a concentration of 1:10 cell/yeast for 6 and 24 hours. The expression levels of apoptotic genes *Bcl-2*, and *caspase-3*compared to untransfected cells was evaluated by Real-Time PCR. Results: caco-2/hBD-2 and -hBD-3 cells showed, after 21 culture days, higher expression levels of occludin, zonulin-1 and claudin-1, and higher TEER values compared to untransfected cells. In addition in transfected cells the invasive ability of *C. albicans* is significatively reduced, as well as the expression levels of genes involved in the apoptotic pathway.

Discussion and conclusions:The study of interaction between antimicrobial peptides and microbiota will allow us in the future to better clarify the mechanisms underlying their role in defense of host against intestinal pathogens. In addition, translational use of AMPs in clinical and other applied settings will be greatly enhanced by understanding how specific AMPs function in their natural contexts and how their evolutionary history may predict their future utility.

117 - A high-throughput platform for the rapid screening of neutralizing antibodies against SARS-CoV-2 virus

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Introduction. As July 24th, 2020, SARS-CoV-2 virus has caused more than 15,000,000 confirmed cases and 600,000 deaths worldwide. The virus spread all over the world leading the World Health Organization to raise the global alert for COVID-19 to the maximum level. Efficient and specific antiviral therapy are urgently needed to face the outbreak of SARS-CoV-2. Plasma therapy has been successfully deployed and others antibody-based strategies represent promising therapeutic options. In this scenario a reliable assay for the rapid detection of neutralizing antibodies is sorely needed.

Materials and methods. Here, we adapted our BSL2 based technology to implement a coronavirus screening assay that can efficiently identify neutralizing antibodies. We show here that our assay can be used as high-throughput platform for the rapid screening of neutralizing antibodies (nAb) in BSL2 conditions. SARS-CoV-2 spike protein (S) represents the main target of nAb. Our cell-based assay mimicking multiple rounds of infection allows the detection of antibodies targeting the SARS-CoV-2 S.

Results. We screened sera from patients with vary degrees of clinical signs (from moderate to severe) for the presence of neutralizing antibodies, in addition to the neutralizing properties we evaluated the binding to the S proteins from clinical isolates as well as from different beta-coronaviruses (e.g. SARS-CoV-1 and MERS) using a cell-based ELISA assay. We show that our assays can also detect cross-reactivity against other coronavirus spike proteins.

Discussion and conclusions. We compared the results obtained in our assays with those on live virus finding a high degree of concordance. Our results suggest that our platform represents a valid instrument for the rapid identification of prophylactic and therapeutic nAb to curb SARS-CoV-2 pandemic.

118 - GT-2.9, a synthetic resveratrol derivative, inhibits herpes simplex virus type 1 (HSV-1) life-cycle in different in vitro HSV-1 infection models

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Introduction. 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. It induces several diseases (i.e. herpes labialis, herpes simplex encephalitis), and experimental studies also suggest a causal link between recurrent HSV-1 infection and neurodegenerative disorders. HSV-1 infections are treated with antiviral agents, such as acyclovir, but immunocompromised patients could need long-term anti-herpetic therapy, inducing drug resistance. Thus, the development of novel anti-HSV-1 treatments is needed. Resveratrol is a natural polyphenolic compound with a range of pharmacological properties, including antiviral activity, but its pharmacokinetic consists of rapid metabolism and poor bioavailability. The synthesis of resveratrol derivatives, in which some functional groups are modified, aims to find molecules with the same or better properties than resveratrol, but with higher bioavailability. Here we investigated the anti-HSV-1 properties of GT-2.9 [(E)-1,1'-(but-2-ene-1,4-diylbis(4-hydroxy-3,1-phenylene)) bis(ethan-1-one)], a synthetic resveratrol derivative.

Materials and Methods. Confluent monolayers of African green monkey (Vero), human embryonic kidney (HEK), neuroblastoma (SH-SY5Y) and glioblastoma (A172) cells, primary murine neurons/astrocytes were infected with HSV-1 (multiplicity of infection of 1) for 24 hrs. Infected cells were subjected to time-of-addition assay with GT-2.9 (40 µg/ml). Viral titers were quantified by standard assays. Viral and cellular protein expression was examined by q-RT-PCR, western blotting and confocal microscope analysis.

Results. Our results demonstrated that GT-2.9 treatment was not cytotoxic (CC_{50}=71 µg/ml), and the molecule showed great antiviral properties against HSV-1 (IC_{50}=2.98 µg/ml; SI=24). GT-2.9 was able to inhibit the post-adsorption phase of HSV-1 life-cycle (more than 2 log inhibition respect to GT-2.9-not treated) by interfering with the immediate-early viral protein production, that are needed for the expression of early and late viral gene products. The antiviral effect of GT-2.9 was associated to the inhibition of the NF-κB signaling pathway and the activation of the transcription factor Nrf2. In addition, when the molecule was added in combination with acyclovir, a synergistic action against HSV-1 infection was observed.

Conclusions. Our *in vitro* data suggest GT-2.9 as a promising resveratrol derivative with anti-HSV-1 activity. In addition, it could be considered for combinatorial drug treatment with nucleoside analogues, such as acyclovir.

120 - Nasopharyngeal expression levels of SARS-CoV-2 receptor ACE2, but not of host protease furin, is more related to interferon activation than to age of infected patients

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide since December 2019.The angiotensin-converting enzyme 2 (ACE2) provides the entry point of SARS-CoV-2 to infect a wide range of human cells. Viral entry into human lung cells is also enhanced through the spike protein activation by the serine protease furin. As ACE2 has been recently demonstrated to be an interferon (IFN)-stimulated gene (ISG), in order to characterize the relation of ACE2 and furin with the IFNs, *in vivo* and *in vitro* experiments were performed.

Materials and Methods: Fifty-nine children with a clinical diagnosis of bronchiolitis and pneumonia and 48 adults with respiratory symptoms attending University Hospital "Policlinico Umberto I" over the 2019/2020 winter season were enrolled. Respiratory specimens (nasopharyngeal washings/swabs) were tested for 14 respiratory viruses and for SARS-CoV-2; remaining aliquots were centrifuged, and RNA extracted from cell pellet for ACE2, furin and ISG15 mRNA quantification through RT-Real time PCR. Moreover, to examine the IFN-mediated activation of ACE2 and furin, cultured A549 (a lung carcinoma derived cell line) were stimulated with natural IFN-alfa at 5x104 IU/mL and IFN-beta-1a at 500 ng/mL. The comparative threshold cycle method was applied to calculate the fold changes of theACE2, furin, and ISG15, ISG56 and IRF-7. Statistical analysis was performed using Stata 15.

Results: Over half of the patients (64/107) presented respiratory viral infection, no one was positive to SARS-CoV-2. A slight increase in ACE2 levels was found in children (<13 years) compared with adults ($p=0.06$) and the ACE2 gene expression level was inversely correlated with age ($r=-0.32$, $p=0.01$) in contrast to adults. Regarding sex and virus detection, no significant difference was evident testing ACE2. Furin expression was positively correlated with ACE2 ($r=0.34$, $p<0.001$), but not significantly related to patients' age. Higher levels of ISG15 were found in children ($p<0.001$). Analyzing possible correlation with ISG15, we found a strict relation for ACE2 ($r= 0.41$, $p<0.001$) but not for furin. Moreover, in *in vitro* experiments, as the ISG stranscription was significantly elevated after the IFN stimulation, the ACE2 levels were also upregulated by IFN-beta (72.8 fold) and alfa (21.5 fold), differently from furin.

Discussion and Conclusions: These preliminary results suggest that the activation of ACE2 *in vivo* is independent from sex and virus infection while the age relation still need investigation, although ACE2 levels paralleled that of ISG15 in the younger patients, the best marker of type I and III IFNs' activation. Consistently, in *in vitro* experiments, ACE2 expression was induced by IFNs.

122 - Human Endogenous Retroviruses activation and cytokines expression in swab samples from COVID-19 Patients

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Introduction: HERVs are genetic elements, relics of infections by exogenous retroviruses that occurred throughout evolution comprising approximately 8% of the human genome. Due to their long co-evolution with humans, some HERVs have been coopted for physiological functions, including gene expression regulation and innate response modulation, while their reactivation in response to external stimuli e.g. viruses, hormones and cytokines has been associated with human pathological conditions, including cancers, diabetes and rheumatoid arthritis, that have been recognized as underlying illnesses in older patients infected by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that develop severe coronavirus disease (COVID-19). The host immune reaction to SARS-CoV-2 infection involves several components of the immune system that appear responsible for viral elimination and recovery from the infection. Nonetheless, such immune responses, including cytokines storm and lymphopenia, are implicated in the disease progression to a more severe form and lethal process. Given HERVs role in modulating host innate immune response, here we aimed to investigate HERVs and cytokines expression in nasopharyngeal swab samples from COVID-19 patients to explore the potential involvement of HERVs in SARS-CoV-2 infection and local immune response of COVID-19 patients.

Materials and methods: residuals of nasopharyngeal/oropharyngeal swabs samples from individual who attending “Tor Vergata” University Hospital of Rome were collected and the expression of several HERVs family (HERV-H, HERV-K HML-2, HERV-W), cytokines (IL-6, IL-1□, IL-10, TNF□) and of the receptor ACE2 have been analyzed by RT-Real time PCR. The non-parametric Mann-Whitney test was used for statistical analysis.

Results: A significant increase of the HERV-K (HML-2) activity in parallel with the higher expression of inflammatory cytokines (IL-6, IL-10 and TNF□) and ACE2 has been observed in SARS-CoV-2 positive swab samples compared to negative samples. Moreover, by Spearman correlation analysis, a positive correlation between HERV-K and cytokines (IL-6, IL-10, TNF□) has been found. The analysis also showed that within the group of SARS-CoV-2 positive individuals, the expression of HERV-K is lower in individuals who were undergo hospitalization than in individuals who are not hospitalized.

Discussion and conclusion: the obtained results show that in SARS-CoV-2 positive swab samples the increase of HERV-K expression runs in parallel with high expression levels of cytokines, known to be involved in the COVID-19 progression. Although preliminary, the data suggest HERVs expression as a potential biomarker of the disease severity and their possible involvement as actors of the mucosal innate immune response in COVID-19 patients.

124 - Interplay between the DNA damage response and NF-KB signaling regulates JCV replication

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Introduction. Infection of glial cells by human neurotropic polyomavirus JC (JCV) causes the demyelinating disease progressive multifocal leukoencephalopathy (PML) and rapidly inflicts damage to cellular DNA. This activates DNA damage response (DDR) signaling including induction of expression of DNA repair factor Rad51. We previously reported that Rad51 co-operates with the transcription factor NF-kB to activate JCV early transcription. DDR is also known to stimulate NF-kB activity which is initiated by Ataxia telangiectasia mutated (ATM) protein, a kinase recruited and activated by DNA double-strand breaks. Downstream of ATM, a series of post-translational modifications of NF-kB essential modulator (NEMO) occurs, resulting in NF-kB activation.

Material and Methods. For NEMO translocation assay, SVGA cells were transfected with Flag tagged NEMO (Flag-NEMO) expression plasmid and infected with JCV Mad1: cytoplasmic and nuclear protein fractions were analyzed by Western Blot (WB). To study the role of ATM phosphorylation in JCV infection, we performed a WB for phosphorylated form of ATM (phospho-ATM) and viral proteins from JCV Mad1-infected SVGA cells; we also checked the effect an ATM inhibitor KU-55933 on JCV infection by WB and quantitative PCR. The interaction between ATM and NEMO was assessed by Immune-Precipitation and the SUMOylation of NEMO were analyzed by WB after over-expression of the viral protein large T antigen (LTAg). Finally, the expression of Rad51 were analyzed by WB at 5, 10 and 15 days post-infection and its effect on JCV non-coding control region (NCCR) were determined by Luciferase assay in presence of mutated variants of NF-kB binding-site on NCCR.

Results. The DNA damage induced by JCV infection has been associated with the induction of DDR, which involves activation of phospho-ATM and an increased expression level of the DNA repair protein Rad51. Furthermore, specific inhibitor of ATM inhibits JCV replication and JCV infection caused a redistribution of NEMO from cytoplasm to nucleus. Co-expression of JCV LTAg and Flag-NEMO showed the occurrence of SUMOylation of NEMO, while co-expression of ATM and Flag-NEMO demonstrated physical association between ATM and NEMO. Importantly, we found that Rad51 complexes with NF-KB p65 subunit, which stimulates JCV transcription.

Discussion and Conclusion. These observations indicate that activated ATM has an important role in coordinating the molecular events involved in DDR after JCV infection, such as the activation of NF-kB by a novel mechanism known as nucleus to cytoplasm or “inside-out” NF-KB signaling. Moreover, the DNA damage inflicted by JCV infection induces the expression of Rad51, which acts together with NF-KB to promote productive infection.

125 - Outer membrane vesicles derived from Klebsiella pneumoniae influence the miRNA expression profile in human bronchial epithelial BEAS-2B cells

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Introduction: *Klebsiella pneumoniae* (*K. pneumoniae*) is an opportunistic pathogen that causes nosocomial and community-acquired infections. *K. pneumoniae* has important virulence factors required to colonization, adherence, invasion and infection's progress. This bacterium, rapidly, acquires antibiotic resistance mechanisms, causing an increasing problem in choosing a valid antibiotic treatment. Given the clinical significance of this pathogen, a better understanding of others mechanism of virulence are fundamental for designing new strategies to treat this infections. *K. pneumoniae*, as Gram negative, release outer membrane vesicles (OMVs). OMVs are a vehicle for the transport of virulence factors to host cells, causing cell injury. Our previous studies have shown changes of gene expression in human bronchial epithelial (BEAS-2B) cells after treatment with *K. pneumoniae* OMVs. The gene expression variations could be regulated through microRNAs (miRNAs), which are involved in several biologic mechanisms. Little is known about the function of miRNAs in the BEAS-2B cells after OMVs interaction. Therefore, the aim of our study was to evaluate the difference of miRNA expression changes after treatment with OMVs produced by standard and field isolated *K. pneumoniae* strain.

Materials and Methods: OMVs from standard and field isolated *K. pneumoniae* strains were purified using ultracentrifugation and filtration. OMVs were quantified using a Bradford assays. The vesicles diameter size and polydispersity index were defined by Dynamic Light Scattering (DLS). BEAS 2B cells were treated with 5µg /ml of OMVs for 6 hours and after miRNA extractions were performed. The expression profiles of microRNA were carry out using 384-well TaqMan Human MicroRNA array. TargetScan, DIANA-microT-CDS and miRTarBase were exploited to predict the target genes of the miRNA dataset. The Metascape software was used for Gene Ontology enrichment analysis. Transcripts levels of four miRNAs were measured by RT-qPCR.

Results: All vesicles were analysed in terms of diameter and size distribution, through DLS. DLS analysis had shown that OMVs from standard strain were characterized by a smaller diameter and slightly heterogeneous size distribution compared to the OMVs from clinical *K. pneumoniae*. In addiction, clinical strains produced more OMVs than the standard one. Microarray analysis and RT-qPCR identified the dysregulation of miR-223, hsa-miR-21, hsa-miR-25 and hsa-let-7g miRNAs sequences. Target gene prediction revealed an essential role of these miRNAs in regulation of host immune responses affecting NF-κB (miR-223), TLR4 (hsa-miR-21), cytokine (hsa-miR-25) and IL-6 (hsa-let-7g miRNA) signaling pathways.

Discussion and Conclusions: Our results suggested an important role of OMVs in the inflammatory response via miRNAs regulation.

127 - Differential respiratory tract expression of SARS-CoV-2 ACE2 receptor and of serine proteases and relationship with IFN response in cystic fibrosis patients

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Introduction: The impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on cystic fibrosis (CF) remains poorly understood, as SARS-CoV-2 infections have been observed in few CF patients and were associated to a not severe infection. Respiratory samples collected from patients during clinical visits at the Regional (Lazio) CF Reference Center from November 2019 through March 2020 were retrospectively analyzed for virological and gene expression investigation.
Materials and Methods: All samples (n=265), residual specimens from routine diagnostic, were tested with RT-PCR for the following viruses: Respiratory syncytial virus (RSV A/B), Influenza A virus (FluA), Rhinovirus (HRV), Metapneumovirus and Coronaviruses, HCoVs OC43, 229E, NL-63 and HUK1. Real-Time PCR reactions targeting the RdRp and the E genes of SARS-CoV-2 was carried out. In addition, gene expression investigation on a subgroup of 46 adult CF patients and a matched group of 45 healthy controls was performed for ACE2, the main SARS-CoV-2 receptor, and furin and TMPRSS2, that enhance the viral entry. Given the ability of IFN-I to induce ACE2, ISG15 gene expression, a well-known marker of IFNs activation, was also assessed. All statistical calculations were performed using SPSS v.20.0.
Results: No SARS-CoV-2 positive result was identified in the respiratory samples analyzed. By contrast, 95/265 samples (35.8%) were positive for 1 or more non–SARS-CoV-2 viruses. Patients were divided in 3 age groups: ≤10 years children (n=43), 11-18 years adolescents (n=39) and >18 years adults (n=183). Children had the highest rate of viral infections (p=0.004). Overall, HRV were the most frequently detected virus (n=56), followed by RSV (n=11), FluA (n=10), HCoV-229E (n=7), HCoV-HKU1 (n=3). Eight coinfections were observed. Regarding gene expression, lower ACE2 levels were found in CF patients compared to the control group (p=0.034). Moreover, a trend toward a lower expression of both furin and TMPRSS2 was also observed in CF patients. CF patients showed higher ISG15 levels compared to healthy controls (p<0.001). A strong positive correlation was found between ISG15-mRNA concentration and ACE2 in both CF patients (r=0.47, p=0.001) and healthy controls (r=0.34, p=0.02).

Discussion and Conclusions: In an early pandemic period, we did not find any case of SARS-CoV-2 in CF patients with stable or variable respiratory signs across a broad age range. In the preliminary analysis of gene-expression in oropharyngeal cells, we confirmed that a relationship between ACE2 levels and IFN-I response does exist also in CF patients. The reduced ACE2, furin and TMPRSS2 expression found in CF patients could be related to the mild SARS-CoV-2 disease profile shown in CF patients.

129 - Thymosin alpha 1 mitigates cytokine storm in blood cells from Covid-19 patients

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Introduction: COVID-19 is a disease distinguished complex alterations of the immune system such as lymphopenia, T cell subsets exhaustion and inflammatory cytokine storm, that have been associated with adverse outcomes in patients, underlining host-microbe interaction as crucial during SARS-CoV-2 infection and COVID-19 progression. Thymosin alpha 1 (Ta1) is one of the molecules recommended in the management of COVID-19, since is able to restore the homeostasis of the immune system in diseases with hindered or impaired response such as infections and cancer. The biological processes modulated by Ta1 in CD8+ T cells under inflammatory condition have been evaluated by an enrichment pathways analysis and the identified genes have been analysed in *ex-vivo* Ta1 treated COVID-19 blood cells.

Material and methods: Metascape was used to identify biological processes regulated by Ta1 in CD8+ T cell +LPS. Blood samples of fifteen SARS-CoV-2 positive individuals attending the Policlinic Hospital of Rome "Tor Vergata" were exposed for 8 hours to 50μg/ml Ta1 and analysed by Real time PCR and Flow Cytometry.

Results: Metascape analysis of CD8+ T cells from Healthy donors (HDs) treated with Tα1+LPS identified several modulated biological pathways related to cytokines network and regulation of immune response against pathogens. Ta1 restored gene expression in CD8+ T cells under inflammatory condition, downregulating biological process related to inflammatory response (IL-17pathway, cytokine/chemokine production and TLRs cascade). Several of the identified genes were upregulated in COVID-19 patient blood cells compared to HDs. The *ex-vivo* treatment with Ta1 significantly down-regulated the transcriptional expression of IL6, TNFa, IL1b and TRAF2 in COVID-19 blood cells, while up-regulated in HDs. Ta1 maintained high levels of IL10, a master regulator of immune responses, both in HDs and COVID-19. Flow cytometry analysis demonstrated higher expression of IL6, CD38 and HLADR activation markers in COVID-19 CD8+ T cells that have been significantly attenuated by Ta1 in patients, but increased in HDs. In CD4+ T cells the activation markers were down-regulated both in COVID-19 patients and HDs after treatment.
Discussion and conclusion The identified genes associated to cytokine signalling and production were found upregulated in blood cells from COVID-19 patients and the *ex-vivo* treatment with Ta1 mitigated cytokines expression and inhibited lymphocytes hyperactivation specifically in COVID-19 CD8+ T cells. Ta1 differentially modulates functional genes to control immune response homeostasis according to the cellular activation state and physiopathological condition, suggesting the potential to modulate immune response homeostasis and restore the cytokine storm *in vivo*.

130 - Key genetic elements single or in clusters underlying geographically-dependent SARS-CoV-2 genetic adaptation and their impact on binding affinity to drugs and immune control

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Background: To define key genetic-elements, single or in clusters, underlying SARS-CoV-2 evolutionary diversification across Continents, and their impact on drug binding-affinity and viral antigenicity.

Methods: 12,150 SARS-CoV-2 sequences (publicly-available) from 69 Countries are analyzed. Mutational clusters are assessed by hierarchical-clustering. Structure-based virtual screening (SBVS) is used to select the best inhibitors of the main Protease (3CL-Pr) and RNA-dependent RNA polymerase (RdRp) among the FDA-approved drugs and to evaluate the impact of the identified mutations on binding-affinity for these drugs. Lastly, the impact of mutations on epitope-recognition is assessed *in silico* through the Immune Epitope Database and Analysis Resource (IEDB), following Grifoni, 2020.

Results: 35 key mutations are identified, all with a prevalence≥0.5% and residing in different viral proteins. 16/35 mutations form tight clusters involving multiple SARS-CoV-2 proteins, highlighting inter-genic co-evolution. Some clusters, including $D614G_{Spike}$+$P323L_{RdRp}$+$R203K_{N}$+$G204R_{N}$, occur in all Continents (bootstrap=1.0). Differently, other clusters show a geographically-restricted circulation: $T1198K_{PL\text{-}Pr}$+$P13L_{N}$+$A97V_{RdRp}$ in Asia (bootstrap=1.0), $L84S_{ORF\text{-}8}$+$S197L_{N}$ in Europe (bootstrap=1.0), $Y541C_{Hel}$+$H504C_{Hel}$+$L84S_{ORF\text{-}8}$ in America and in Oceania (bootstrap=1.0 and 0.97, respectively).
SBVS identifies 20 best RdRp inhibitors and 21 best 3CL-Pr inhibitors belonging to different drug-classes. Notably, mutations in RdRp or 3CL-Pr modulate positively or negatively the binding-affinity of these drugs. Among them, $P323L_{RdRp}$ (prevalence:61.9%) reduces the binding-affinity of specific compounds including remdesivir (G-score: -8.80 vs -9.91 kcal/mol for $P323L_{RdR}$ vs *wt*). Conversely, $P323L_{RdRp}$ determines an increase of the binding-affinity of the purine analogues penciclovir and tenofovir (G-score: -10.12 vs -8.22 kcal/mol and -8.45 vs -8.25 for $P323L_{RdR}$ vs *wt*, respectively), suggesting a potential hypersusceptibility to these drug-candidates in presence of this mutation. Finally, specific mutations hamper (up to abrogate as for $Y541C_{Hel}$+$H504C_{Hel}$) Class-I/II epitopes recognition, while $D614G_{spike}$ profoundly alters the structural-stability of the recently-identified B-cell epitope encompassing amino acids 592-620 of Spike protein.

Conclusions: Key genetic-elements reflect geographically-dependent SARS-CoV-2 genetic adaptation, and can play a role in modulating drug-susceptibility and in hampering viral-antigenicity. Thus, a close monitoring of SARS-CoV-2 mutational patterns is crucial to ensure the effectiveness of treatments and vaccines worldwide.

131 - Dysregulation of interferon stimulated gene response in the respiratory tract of patients with coronavirus disease 2019 (COVID-19)

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become the most severe public healthcare concern. Interferon (IFN) represents a critical, first-line defense to infection and injury as part of innate immunity. The influence of type I and III IFN pathway in the respiratory tract infection in patients with 2019-nCoV is still poorly known. We evaluated the respiratory tract expression of the genes encoding IFN-alfa, IFN-beta, IFN-lambda 1-3, IRF-7 and of IFN stimulated genes (ISGs), such as ISG15 and ISG56, to delineate IFN signatures and impact to the pathogenesis of SARS-CoV-2.

Materials and Methods: Oropharyngeal swabs were collected from 54 patients with symptomatic SARS-CoV-2 infection hospitalized at the Policlinico Umberto I Hospital in Rome and from 29 negative healthcare workers. Respiratory samples were divided into two aliquots: one was treated for SARS-CoV-2 detection using RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona diagnostics, Germany); in the second aliquot total RNA was extracted from cell pellet and then analyzed for gene expression of IFN-alfa, IFN-beta, IFN-lambda 1-3, IRF7, ISG15, and ISG56 through RT/Real Time PCR. The housekeeping gene beta-glucuronidase was used as an internal control. Gene expression values were calculated by the comparative 2^-deltaCt methods. Clinical data were available for 42 out of 54 SARS-CoV-2 positive patients analyzed. Statistical analysis was performed using SPSS v.20.0.

Results: Overall IFN-alfa, IFN-beta, IFN-lambda 1-3, IRF7, and ISGs mRNA levels in oropharyngeal swabs were significantly increased in SARS-CoV-2 infected patients compared to those detected in healthcare workers. SARS-CoV-2 threshold cycle (Ct) values negatively correlated to ISG15, ISG56 and IRF7 mRNAs levels (ISG15 r=-0.3066, p=0.0405; ISG56 r=-0.3672, p=0.0182; IRF7 r=-0.3733, p=0.0192). Interestingly, a small subgroup of 6 patients who were supported by invasive mechanical ventilation showed a general decrease in the expression of some IFN genes with a significant lower level of ISG15 and ISG56, compared to the patients who do not required oxygen support and those who received non-invasive ventilation ($p<0.05$; $p<0.05$).

Discussion and Conclusions: Our preliminary results suggest a differential IFN-I/III activity in the respiratory tract of SARS-CoV-2 patients, depending on development of immunopathology and severe disease. We showed that type I/III interferons (IFN), IFN-regulatory factor 7 (IRF-7), and IFN stimulated genes (ISGs), are highly expressed in the oropharyngeal swabs of SARS-CoV-2 positive patients compared to health controls. Notably, the subgroup of critically-ill patients that required invasive mechanical ventilation had a general decrease in expression of IFN/ISG genes.

134 - AN UNUSUAL CASE OF CUTANEOUS LEISHMANIOSIS

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Introduction: Leishmaniasis is a vector-borne protozoan infection of which clinical range spectrum from asymptomatic infection to fatal visceral leishmaniosis. Sicily is considered to be a sub-endemic area for cutaneous leishmaniosis. *L. infantum* is the most common species on the island. Clinical examination of suspected cases, parasitological diagnosis by skin scraping test and immunodiagnosis are the routine methods available for the diagnosis of leishmaniasis. Monoclonal antibodies have long been available for the identification of *Leishmania* species but they are not frequently used.

Materials and Methods: 46 y.o. male patient, with multiple ulcerative lesions in the breast area including areola; axillary lymphadenopathy; screening for breast cancer. **Results**: Analysis of the data provided by haematochemical and anatomopathological examinations, led us to investigate in the direction of leishmaniasis.

Discussion and **Conclusions**: Molecular techniques in diagnosis of leishmaniasis has become increasingly relevant due to their remarkable sensitivity, specificity and possible application to a variety of clinical samples. Among these, real-time PCR has become increasingly popular in the last years, not only for detection and quantification of *Leishmania species*. However, despite RT-PCR methods which were proven to be very effective in the diagnosis of leishmaniasis, a standardized method does not yet exist. Dermoscopy, is a non-invasive evaluation that help to visualize the inner part the cutis (epidermis and dermis). Furtheremore, there has been increasing evidence that dermoscopy can also be useful in the diagnosis of skin infections. Dermoscopy is a promising non-invasive tool useful to predict the clinical course in cases of cutaneous leishmaniasis and the patient's response to the therapy.

139 - Impact of SARS-CoV-2 infection on oral microbiota and local immune response

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Introduction. The presence of SARS-CoV-2 has been recently demonstrated in the sputum or "posterior oropharyngeal saliva", suggesting that the oral mucosa supports the infection and that the shedding of viral RNA outlasts the end of symptoms. Recent transcriptome data have shown that ACE2 and TMPRSS2 are expressed on oral cavity mucosa, suggesting a potentially high vulnerability for SARS-CoV-2 infection and a worth studying aspect of COVID-19 pathogenesis. In the present study, for the first time, we demonstrate the impact of SARS-CoV-2 infection on oral microbiota composition and inflammatory profile.

Materials and Methods. Hospitalized COVID-19 patients (n=26, mean age 66±16 years) and matched healthy controls (n=13) were enrolled. For each patient, an oral swab and a serum sample were retrieved. Microbiota structure was analyzed by 16S rRNA-V2 region targeted sequencing, using the Ion Torrent PGM platform. Oral and seric concentrations of 27 cytokines were assessed using magnetic bead-based multiplex immunoassays, using the Bio-Plex 200 instrument. Dedicated bioinformatic pipelines and multivariate statistics coupled to network analysis were employed to cross-relate all datasets.

Results. A significant diminution in species richness was observed in COVID-19 patients, along with a marked difference in beta-diversity. Species such as *Prevotella salivae* and *Veillonella infantium* were distinctive for COVID-19, while *Neisseria perflava* and *Granulicatella elegans* were predominant in controls. Interestingly, these two groups of oral species oppositely clustered within the network, defining two distinct Species Interacting Group (SIGs). A bunch of cytokines (TNFα, IL15, IL6, IL5) were distinctive for COVID-19, while only IL12p70 emerged in controls. We also defined a specific bacterial consortium able to counteract COVID-19-related oral cytokines, following a novel index, called C4, firstly proposed here. The oral COVID-19 cytokine profile, involved in early inflammatory response, also correlated with serum cytokine pattern.

Discussion and Conclusions. This pilot study evidenced a distinctive oral microbiota composition in COVID-19 subjects, with a definite structural network in relation to secreted cytokines. Our results would pave the way for a theranostic approach in fighting COVID-19, trying to enlight the intimate relationship among microbiota and SARS-CoV-2 infection.

5 INNOVAZIONI IN DIAGNOSTICA

11 - Impact of urogenital infections on Sperm DNA Fragmentation and seminal parameters

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Introduction: Male infertility, found in 7% of worldwide population, is the basis of 50% of couple infertility cases. There are known pre-testicular, testicular and post-testicular causes which interfere with the spermatozoa features. In the other cases, in which the cause of infertility remains unexplained, it is assumed that this condition is due to the coexistence of different factors, such as, genetic disorders, environmental pollution and infections. The latter factor has a negative impact on sperm bio-functional competence, especially if there was a prolonged exposure to one or several microbial noxae on one or more male accessory sex glands. To date, a higher percentage of Sperm DNA Fragmentation (SDF) has been found in infertile subjects (>30%) than those found in fertile subjects (5-15%). The aim of the present study was to assess the impact of urogenital infections on SDF and seminal parameters.

Materials and Methods: 53 semen samples from men (27-44 years) undergoing fertility investigations were included, based on specific esclusion criteria. Prior the sampling, the patients proceeded with urine collection in order to better differentiate the infection of the seminal tract from that of urinary tract, then standard semen analysis was carried out according to reference protocol, and the infectious status was determined in accordance with standard culture methods for spermiocolture analysis. The SDF analysis was carried out by TUNEL assay.

Results: The standard semen analysis showed parameters of inflammation in 70% (37/53) of the semen samples analyzed of which 70% of these were infected. The sperm parameters between patients with and without inflammation were significantly different. Moreover, the infectious status was inversely correlated with motility parameters and directly correlated with the percentage of non mobile spematozoa. The samples with inflammation traits show that 43.2% (16/37) has %SDF greater than 30%, 35% (13/37) has %SDF between 15-30%. Similarly, the %SDF exhibited a negative correlation with total motility parameter and a positive correlation with the percentage of non mobile spematozoa in samples with inflammation signs.

Discussion and Conclusions: Urogenital infections and the %SDF significantly affect the seminal fluid parameters, in particular these parameters negatively influence the sperm motility, essential for the fertility trait of seminal fluid, while, positively affect the percentage of non mobile spematozoa. Therefore, in the diagnostic path for the evaluation of infertility, the presence of an inflammatory state of the seminal fluid, associated with reduced motility of the spermatozoa and increased presence of immobile forms, could suggest the presence of an ongoing infection and a high degree of SDF.

18 - To standardize and modernize: a novel approach in TB diagnosis.

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Introduction: World Health Organization (WHO) estimates that about one fourth of the world's population is infected by *Mycobacterium Tubercolosis* and about 5-10% of those develop tuberculosis (TB). A total of 1.5 million people died from TB in 2018, and it can be easily spread through the air. If there are evidence, or suspects, the patient has to be tested and sputum smear microscopy, with a sensitivity of 30% up to 60%, and culture that remains the gold standard to diagnose TB. If TB diagnosis, result must carry the number of bacteria found in the sample and their morphological characteristics permits understanding whether the therapy is having beneficial effects on the patient or not.

Materials and Methods: We analyzed sputum smear samples for sample screenings related to a negative culture for confirmation. We used a dedicated high-resolution software, linked to a fully automated scanning microscope, to collect images using a 20x objective with Aurammine stained slides, and 40x-oil objective with Kynioun stained slides together with a high-resolution color camera. A pre-scan automatically identified the sample on the slide, then the system divided the sample into different field of views (FOVs) and collected images from 100 fields across the smear.

Results: After 2 weeks, we demonstrate that the system can identify relevant objects from all samples. Since the negative samples are the most critical ones, the system can also collect false positive objects that the user can confirm or not as a real mycobacteria by checking it using the microscope or switching the focal plane on screen. Since it saves every single object with its coordinates, during the objects confirmation the user can monitor the result of the analysis and the software helps in diagnosis. The tested slide scanning system can scan and classify one slide in about 7 minutes. In this scenario, the analysis becomes more reliable and is not submitted to subjective interpretation.

Discussion and Conclusions: The system can facilitate diagnosis in samples for the research of the *Mycobacterium tuberculosis*. The automated slide scanning saves working time and improves the turnaround time of samples, optimizing laboratory routine. The software leads to a higher safety in sample analysis and reporting related to a standardized protocol for sample processing. In addition, data and images storage into a database becomes very important for data traceability and several further aspects, i.e. second opinion, patient history, teaching activities and to monitor the analysis itself. All these aspects improve the reliability and the workflow of the laboratory.

30 - Clinical validation of a freeze-dried multiplex One-Step RT-qPCR assay for SARS-CoV-2 detection in clinical samples

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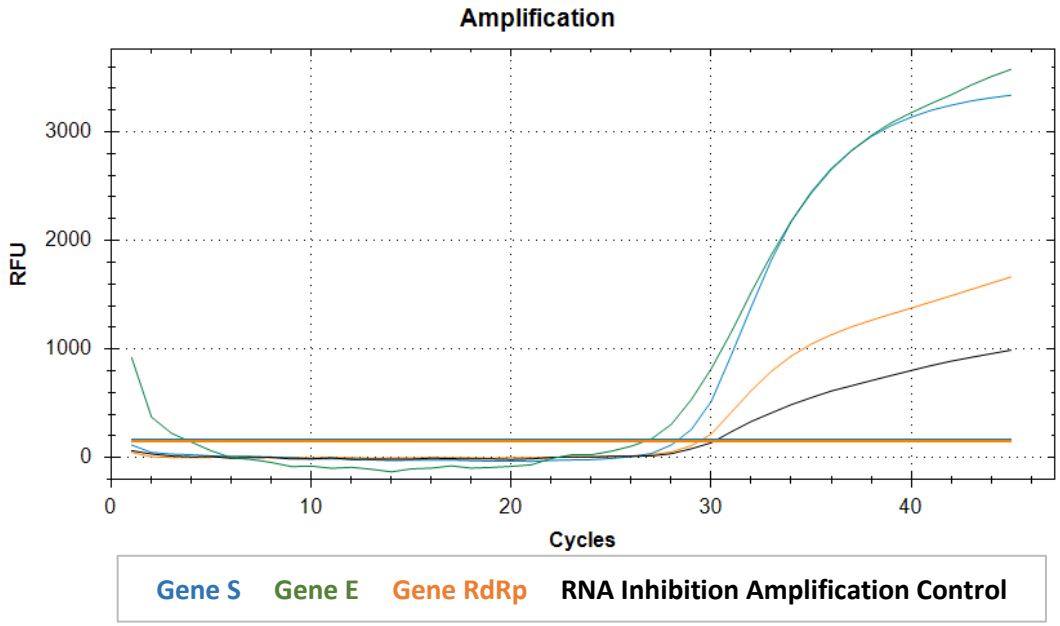
Introduction: In December 2019 a cluster of severe pneumonia emerged in China and it is still spreading all over the world. This pandemic disease has been defined as coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Many of these cases occur also in low-resource countries, where COVID-19 management is hampered by many limitations. Rapid detection is crucial for timely infection identification. The gold standard for the SARS-CoV-2 diagnosis is reverse transcription polymerase chain reaction (RT-qPCR) on respiratory tract specimens. Bioside developed a freeze-dried multiplex One-Step RT-qPCR assay with room temperature (RT) storage to detect SARS-CoV-2 from nasopharyngeal swabs, leveraging the proprietary freeze-drying technology. The objective of this study was to validate the performance of this new CE-IVD assay for the concurrent detection of SARS-CoV-2 and Sarbecovirus in clinical samples.
Materials and Methods: According to World Health Organization guidelines, we developed qualyfast®SARS-CoV-2 Multiplex 2, a freeze-dried assay for detection of four targets, two specific for SARS-CoV-2 (gene S and gene RdRp, detected in two different channel), one for Sarbecovirus (gene E) and one as RNA Inhibition Amplification Control. In this study 215 nasopharyngeal swabs were analyzed; the genomic extraction was performed using both silica-based columns and magnetic beads and the RNA was analyzed with qualyfast®SARS-CoV-2 Multiplex 2. Study analysis and stability tests were performed on 3 different batches.
Results: qualyfast®SARS-CoV-2 Multiplex 2, validated with Joint Research Centre standards, allows simultaneous detection and discrimination of SARS-CoV-2 and Sarbecovirus in positive samples (see graph), with Limit of Detection of 3-10 copies/reaction for both SARS-CoV-2 and Sarbecovirus. The assay



shows 100% specificity and 97.98% sensitivity for SARS-CoV-2 (see table). Shelf-life testing confirmed the freeze-dried format performance up to 12 months.

		True Disease Status	
		Diseased	Non-diseased
Test result	Positive	97 True positive	0 False Positive
	Negative	2 False Negative	116 True Negative

Total	% Sensitivity	97,98
	% Specificity	100

Discussion and Conclusions: This new CE-IVD RT-qPCR assay accurately detected positive samples for SARS-CoV-2 and Sarbecovirus with 100% specificity and a 12 months RT storage stability. This freeze-dried assay is easy to use, fast and suitable device for SARS-CoV-2 and Sarbecovirus detection in clinical specimens. It reduces analytical costs and time of analysis, generates less laboratory environmental wastes and improves patient prognosis. In addition, it is able to integrate standard laboratory molecular biology tests in order to guarantee a large-scale population screening, especially in areas with limited technological/instrumental resources.

42 - Evaluation of asymptomatic SARS-CoV2 infections in low incidence geographic area.

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Introduction: Understanding the prevalence of asymptomatic individuals infected by SARS-CoV2 is of crucial relevance, mostly in view of a possible resurgence of pandemic in the coming seasons. Indeed, while it is relatively easy to isolate Covid-19 symptomatic cases, preventing them from infecting other individuals, it is problematic to control the spread of the disease by infected but symptom-free individuals. This study is aimed to estimate the percentage of asymptomatic SARS-CoV2 infection in a geographic area with relatively low incidence of Covid-19.

Material and Methods: We have recruited 388 healthy volunteers (HV) aged between 19 - 68 years, who declared that they have had none of the symptoms frequently associated with the infection, such as fever, cough, etc., in the past five months. We also examined serum samples from 13 symptomatic patients (SP), 7 of which hospitalized in the University Hospital Federico II. Blood serum samples were analyzed for the presence of anti-SARS-CoV2 IgG by using an ELISA assay based on recombinant viral nucleocapsid protein.

Results: We found that 7 out of 388 healthy volunteers, who declared no symptoms of Covid-19, have positivity values of anti-SARS-CoV2 IgG. In 4/4 subjects with positive values, a second serum sample, drawn 30 days after the previous one, confirmed the positivity. All the 13 SP had values higher than 11 Units (positivity threshold value). In addition, we also performed an unbiased calculation of the changepoint(s) in the entire list of ELISA results (n=405), by using the R package Changepoint. The results suggest that if we consider the positivity threshold indicated by the Manufacturer, the percentage of asymptomatic subjects with bona-fide anti-SARS-CoV2 IgG is of 1.8 (95% confidence interval: 0.69–2.91%). The percentages of HV positivity calculated on the basis of the changepoint analysis are: 0.25% in the high-level range (95% confidence interval: 0-0.66%) and 3.09% in the intermediate-level range (95% confidence interval: 1.65-4.53%).

Discussion and Conclusions: On July 7, 2020, in the Campania region, 4,747 cases of positivity for the presence of the virus in nasopharyngeal swabs have been ascertained on 145,538 subjects examined. Given that social distancing rules have been imposed since 9 March 2020, and that the first Covid-19 cases in Campania were diagnosed on March the 2nd, it is reasonable that the HV recruited have been exposed to possible contagion without restrictions for few weeks. Considering that the population of the examined range of ages in the Campania Region is of about 3.85 million, the estimated range of asymptomatic individuals with anti-SARS-CoV2 IgG should be between 26,565 and 112,350 that are much more than the 4,665 symptomatic diagnosed cases.

45 - Is Droplet digital PCR the best sensitive assay for the SARS-CoV-2 detection?

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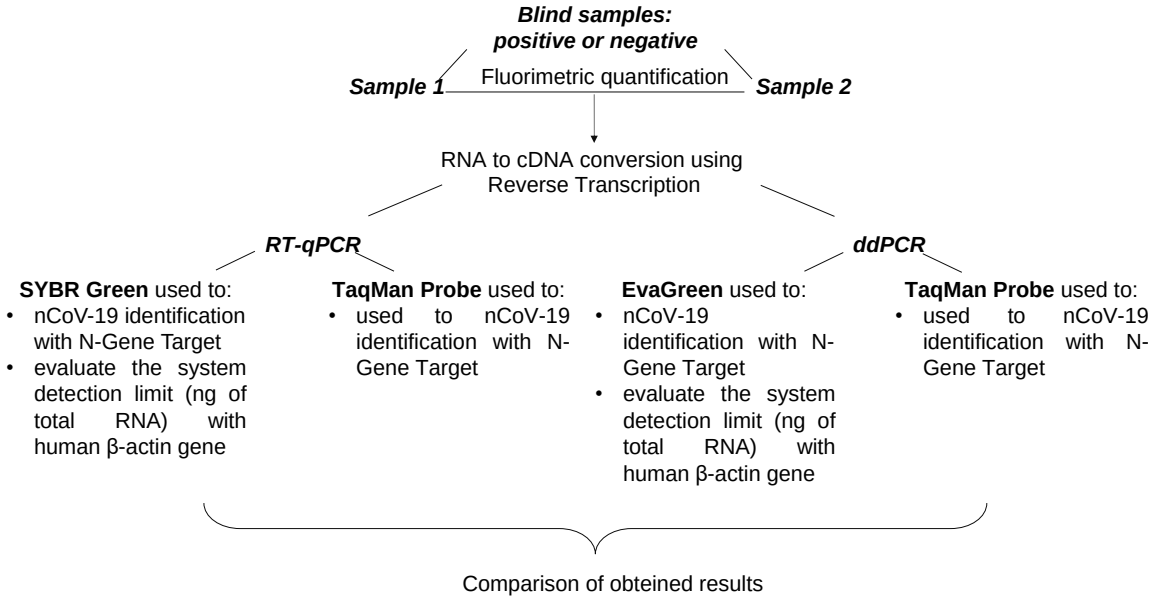
Introduction: The health emergency caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus), represents one of the greatest health and social challenges ever faced worldwide. Since its first outbreak in China in the late 2019, the characteristics of this epidemic have been controversial due to limited information available and the absence of health protocol proven effective in containing or monitoring the spread of this infection. According to the World Health Organization (WHO), the current gold standard method for the diagnosis of SARS-CoV-2 infection is based on the reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The currently most used gene targets are Orf1Ab, Nucleocapsid protein gene and Spike Protein, used for single- or multiplex RT-qPCR. Due to pre-analytical and technical limitations, samples with low viral load are often misdiagnosed as false-negative samples. Therefore, it is important to evaluate other strategies able to overcome the limits of RT-qPCR. The aim of the study was to propose a novel high-sensitive method for the effective detection of SARS-CoV-2 in patients with low viral load, for this reason blinded swab samples from two individuals diagnosed positive and negative for COVID-19 were analysed by RT-qPCR and droplet digital PCR (ddPCR) in order to assess the sensitivity of both methods.

Materials/methods: RNA samples extracted from a negative and a positive rhino-pharyngeal swabs were preliminary tested with a commercial platform. The concentration of total RNA was determined by using a fluorometric assay. For the detection of SARS-CoV-2, the CDC-validated 2019-nCoV_N1 primers and probe were used. The same primers with or without probe were used for the RT-qPCR performed with SYBR-Green and TaqMan probe, respectively. Similarly, both EvaGreen and Probe ddPCR-based methods were used with the same primers and probe to assess ddPCR sensitivity. To confirm the positive signals obtained with ddPCR, the positive droplets were extracted and sequenced.

Results: SYBR-Green RT-qPCR is not able to diagnose as positive samples with low viral load, while, TaqMan Probe RT-qPCR gave positive signals at very late Ct values. On the contrary, ddPCR showed higher sensitivity rate compared to RT-qPCR and both EvaGreen and probe ddPCR were able to recognize the sample with low viral load as positive even at 10-fold diluted concentration.

Conclusions: ddPCR shows higher sensitivity and specificity compared to RT-qPCR for the diagnosis of COVID-19 infection in false-negative samples with low viral load. Therefore, ddPCR is strongly recommended in clinical practice for the diagnosis of COVID-19 and the follow-up of positive patients until complete remission.

Schematic workflow of RT-qPCR and ddPCR experiments.



74 - Neutralizing antibodies against SARS-CoV- 2: comparative analysis of different serological techniques

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Introduction. The pandemic caused by SARS-CoV-2 is a public health emergency of international concern. Administration of heterologous neutralizing antibodies (NAbs) against SARS-CoV-2 is considered a promising strategy for the therapeutic treatment of the coronavirus disease 2019 (COVID-19).

Materials and methods. In this study, we evaluated more than 200 convalescent plasma for the titre of neutralizing antibodies, using an in-house method. The serum neutralization test was performed in microtitre plates, based on the limit diluition method and took 3 to 5 days to complete. A clinical isolate of SARS-CoV-2 was used to detect the neutralization of the virus. Commercial serological methods, produced by Euroimmune, Abbott, Ortho and Diasorin, based on enzyme-linked immunosorbent assay (ELISA) and Chemiluminescence immunoassay (CLIA), were used as indicated by the manufacturer.

Results. The results indicate that about 50% of the tested plasma have a neutralizing antibody titre greater than or equal to 1:80. Moreover, a correlation between value obtained with serum neutralization test and values obtained with the commercial methods was found.

Discussion and Conclusions. SARS-CoV-2 epidemic continues to spread globally and has no specific cure. Development of effective NAbs as therapeutic agents to treat the infection is therefore crucial. Our results established a cut-off for each serological method examined and permits to study the role and significance of NAbs in the course of disease with the use of handy and automated systems.

78 - SARS-CoV-2 LABORATORY diagnosis: The PISA VIROLOGY DIVISION EXPERIENCE

Giovanni Segatori, Fabrizio Maggi, Paola Mazzetti, Anna Lisa Capria, Maria Linda Vatteroni, Veronica Dovere, Cesira Giordano, Alessandro Leonildi, Alessandra Vecchione, Melissa Menichini, Mauro Pistello, and Pisa SARS-CoV-2 working group

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Introduction: Coronavirus disease 2019 (COVID-19) is a disease caused by a novel coronavirus denominated as severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), originated from the town of Wuhan, China, which has rapidly expanded worldwide. In Italy, the first official case of SARS-CoV-2 infection was reported on February 20, 2020; since then the virus has spread very quickly across the country killing over 34,000 people and infecting over 245,000. At July 23, 2020, 10,394 cases of infection have been reported in Tuscany. The Virology Unit of AOUP has been appointed since mid of February 2020 as Regional Reference Centre for the diagnosis of SARS-CoV-2 infection.

Materials and Methods: A total of 47,625 samples (of which 46,461 nasopharyngeal swabs) were obtained from outpatients and inpatients of the Area Vasta Nord Ovest, Tuscany in the period March-May. After viral RNA extraction, all samples were tested for SARS-CoV-2 RNA presence of by one of three different real-time PCR assays (Cobas® 6800 Roche, GeneXpert® Cepheid, Seegene NIMBUS IVD® & STARlet IVD® Arrow Diagnostics). Amplification tests were conducted targeting specific regions of SARS-CoV-2 (ORF1ab, RdrP gene, N gene, E gene).

Results: Overall, 4,834 (10%) samples were positive for SARS-CoV-2 RNA, with the peak of prevalence detected in March (24%). The highest prevalence was found in the 70-80 years age group (13%), followed by the over 90 age group (12%). A high prevalence of SARS-CoV-2 infections was also observed in young people less twenty years old (12%). Males were statistically more infected than females (12 vs 9%). Finally, the analysis of the threshold PCR cycles revealed quantitative differences among the samples obtained in the different periods of observation.

Conclusions: a significant number of tests was processed in Pisa Virology laboratory during the first three months of SARS-CoV-2 pandemic. The analysis of our results may be useful for describing the epidemiological trend of SARS-CoV-2 infection in our country.

82 - Molecular validation of pathogen-reduction technologies using rolling-circle amplification coupled with real-time PCR for Torquetenovirus DNA quantification

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Introduction: Pathogen reduction technologies (PRT) based on nucleic-acid damaging chemicals and/or irradiation are increasingly being used to increase safety of blood components against emerging pathogens, such as convalescent plasma in the ongoing COVID-19 pandemic. Quantitative real-time PCR is the current pathogen detection method but, due to the high likelihood of detecting nonviable fragments, requires downstream pathogen culture.

Materials and Methods*:* We report a conservative and easy-to-setup protocol for molecular validation of the Intercept PRT based on the ubiquitous human symbiont Torquetenovirus (TTV) and rolling circle amplification (RCA).

Results: Four of 22 samples were selected based on their TTV drop between pre- and post-PRT having a variation of viremia > 0.5 Log (sample 1600, and 1693) and < 0.5 Log (sample 2586, and 3288), respectively. When these samples were first amplified by RCA, the following TTV quantification by real-time PCR very significantly differed between pre- and post-PRT plasma samples. Of the 4 post-PRT samples, 2 tested negatives for TTV DNA with a load reduction of more than 8.0 and 10.0 Log, respectively, relative to the corresponding pre-PRT samples. The other 2 post-PRT samples remained virus positive, but TTV dropped of about 9.0 Log in the sample 2586 and of 3.0 Log in the sample 1693. An inverse PCR method used for amplifying TTV DNA revealed TTV as full-length genome (about 3.8 kb) in pre- but not in post-PRT sample 1693.

Discussion and Conclusions: The results demonstrate that the *in-house* validation of PRT can be run conservatively without sacrificing previous blood units (such as convalescent plasma units), using a commercially available PCR kits and several modified PCR that can be easily setup at every microbiology laboratory, without need for BSL3 facilities and skilled personnel for pathogen manipulation, spiking and cell culture.

83 - Environmental surveillance of SARS-CoV-2 circulation in Sicily

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Introduction: Italy is among the European countries most affected by the COVID-19 pandemic. During this public health emergency many reports described the detection of SARS-CoV-2 RNA in wastewater around the world. In June 2020, a surveillance project was proposed to assess the presence of SARS-CoV-2 in wastewater in Italy (SARI, Sorveglianza Acque Reflue in Italia), adapting the standard WHO procedure for Poliovirus environmental surveillance. Under the technical-scientific coordination of the Istituto Superiore di Sanità (ISS) the SARI project will construct a network of territorial structures for monitoring the presence of SARS-COV-2 genome in wastewater. The Sub-National Reference Laboratory (SNRL) at the PROMISE Department of Palermo University was already included in the global surveillance network for polio eradication, collecting wastewater samples from Municipal Treatment Plants and Centers for Asylum Seekers (CARA). The Sicilian Poliovirus environmental surveillance network has been integrated in the SARI project in order to timely detect any eventual novel introduction of SARS-CoV-2 in Sicily.
Materials and methods: Wastewater samples are being collected for virological investigations according to standard protocols approved by the WHO, including clarification and concentration of samples (centrifugation and sedimentation in dextran/PEG gradient) and molecular analysis performed by Real Time RT-PCR and nested RT-PCR with primer sets specific for SARS-CoV-2. All positive specimens will be genotyped by sequence analyses in the diagnostic region included in ORF-1ab genome portion.
Results: A prospective environmental sampling campaign, providing for the collection of wastewater samples for virological analysis twice a month, started in June 2020. The Sicilian environmental surveillance network includes 6 Wastewater Treatment Plants: Piana degli Albanesi (PA), Catania (Pantano D'Arci), Messina (Mili), Siracusa, Augusta (SR) and Trapani; 2 Hotspots for migrants: Rosolini (SR) and Pozzallo (RG); 2 Reception Centers for Asylum Seekers (CARA): Milo (TP) and Pian del Lago (CL); and 4 touristic areas: Balestrate (PA), Selinunte (TP), San Vito Lo Capo (TP), and Avola (SR).
Discussions and conclusions: Sewage surveillance would provide early detection of a SARS-CoV-2 circulation, possibly revealing also mild, subclinical, or asymptomatic infections in the population. A wide environmental surveillance network will allow to better define, prepare and coordinate the actions to be taken in the unfortunate event of a second wave of the SARS-COV-2 epidemic in Italy.

104 - Evaluation of the new BCID2 panel: the experience of P.O. Cotugno - Microbiology and Virology laboratory

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Introduction

Sepsis is a serious condition that can lead to severe complications, so directing patients to targeted therapy quickly is essential. Definitive identification of a pathogen can take 24 to 72 hours with traditional culture methods. Delays can lead to inadequate or too broad spectrum antimicrobial therapy and consequent therapy-related complications, antibiotic resistance and increases in patient morbidity, mortality rates and costs. Molecular diagnostic assays have been developed for rapid pathogen identification using flagged blood culture. This approach does not involve bacterial subculture, which enables rapid and accurate pathogen identification.

Materials and Methods

The BioFire FilmArray Blood Culture Identification 2 (BCID2) panel is an automated nested multiplex PCR system that, expanding the BCID panel, enables the simultaneous detection of 43 targets, including 26 bacteria, 7 Fungal pathogens, and 10 antibiotic resistance genes within 1 h from positively flagged blood cultures. In this study, P.O. Cotugno - Microbiology and Virology laboratory (AORN dei Colli) reported results obtained from positive blood culture bottles using the FilmArray BCID 2 panel compared with those obtained using the conventional phenotypic identification methods from sub-cultured colonies, MALDI-TOF and VITEK 2.

Results

Of the 30 positive hemocultures analyzed with FilmArray BCID2 panel, 25 were fully concordant with the results of conventional phenotypic identification methods. Of 5 sample that exhibited discordant results, 2 showed differences for identification of microorganisms not included in the panel BCID 2; for two samples, in culture, two germs (Acinetobacter, Candida parapsilosis) were identified which were not identified with the BCID2 panel. In another sample, BCID2 Panel identified a germ not found in culture. Furthermore, in a sample, a non-CTX ESBL not included in the BCID2 panel was detected by VITEK 2.

Discussion and Conclusions

Notwithstanding the partial non –concordances with traditional tests, the BioFire BCID2 Panel with an expanded menu, is expected to provide rapid and accurate results for key pathogens associated with systemic infections, as well as important AMR markers. In addition, in 8 positive blood cultures, BCID2 allowed us to discriminate between E. feacalis and E. faecium, considered an important hospital pathogens, helping the clinician to rapidly direct therapy.

105 - SARS-CoV-2 RNA Positivity in Recovered COVID-19 Patients Is Due to Low Levels of Non-Replicating Virus

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Background: The follow-up of COVID-19 recovered patients is especially important to assess their infectivity and/or transmissibility statuses in order to maximize the COVID-19 management and containment. The aim of this study was to determine both total (genomic) and replicative (sub-genomic) SARS-CoV-2 RNA levels in nasal/oropharyngeal swab (NOS) samples from patients at follow-up times after COVID-19 recovering.

Materials/methods: We tested 176 NOS samples of COVID-19 recovered patients who were followed up at the Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome from 21 April to 18 June 2020, according to our COVID-19 care protocol. The RT-PCR tests were performed using the Allplex™ 2019-nCoV and the Quanty COVID-19 assays (for total RNA detection and quantification, respectively) and an in-house assay (for replicative RNA detection).

Results: Of 176 NOS samples studied, 32 (18.2%) tested positive for total RNA, with C_T values ranging from 29.3 to 38.8 for E, RdRP, and N genes (9 samples), 32.2 to 39.3 for RdRP and N genes (7 samples) or 35.8 to 39.8 for the N gene (16 samples). Consistently, viral loads ranged from 1.6×10^1 to 1.3×10^4 RNA copies/mL. Interestingly, we found replicative RNA in only one of 32 positive samples based on the presence of E-gene sub-genomic RNA (C_T value of 39.1). The C_T value (29.3) of E-gene genomic RNA in this sample was the lowest among the C_T values of all 9 samples in which the E gene was detected. Testing samples obtained from the 32 patients at the time of COVID-19 diagnosis showed that the C_T values ranged from 17.1 to 38.1 for E, RdRP, and N genes. Of note, the mean C_T value of E-gene sub-genomic RNA (34.9) in these samples differed of 9.0 ± 2.8 from the mean C_T value of E-gene genomic RNA (25.9). Finally, all but one of the 32 patients had positive serology results against SARS-CoV-2.

Conclusions: Our findings show that at least a proportion of COVID-19 recovered patients were still positive for SARS-CoV-2 RNA, despite to a lower extent, and that only a minority of them was likely to have actively replicating virus in the upper respiratory tract.

106 - Validation of the novel FluoroType Mycobacteria assay for the detection and identification of tubercular and non-tubercular mycobacteria

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Introduction. The genus *Mycobacterium* includes many species that can be divided into three large groups: *Mycobacterium tuberculosis complex (*MTBC) cause of tuberculosis, one of the major health issue worldwide, *Mycobacterium leprae* and non-tubercular mycobacteria (NTM) opportunistic species widely distributed in nature, which mainly cause respiratory tract infections. In the last few years in medium- and high-income countries, the incidence of NTM infections has constantly increased in parallel with immunocompromised population, while tuberculosis is more prevalent in low-income countries. For these reasons, specific and rapid diagnostic tools for detection and species identification of MTBC and NTM are urgently needed. The FluoroType Mycobacteria assay (FTMA) (Hain, Germany) is a molecular genetic assay based on the innovative LiquidArray technology which allows detection of MTBC and differentiation at the species level of most relevant clinical NTM in only one tube.

Materials and Methods. 78 prospectively collected routine culture samples seeded both in liquid and solid culture and 54 archived liquid culture samples were included in the study. Samples were extracted by FTMA within 7 days from positivization (on BD BACTEC™ MGIT™ 960 for liquid cultures or by visual inspection on solid cultures) and analyzed with the FluoroType assay on the FluoroCycler XT. The results, available in 2.5 hours including extraction and analysis time, were compared with GenoType Mycobacteria CM/AS, GenoType NTM-DR, and/or genetic sequencing of specific housekeeping genes. Results were analyzed using FluoroType specific software.

Results. The FluoroType assay identification showed concordance rate of 100% for (10) *Mycobacterium abscessus abscessus*, (13) *Mycobacterium abscessus bolletii*, (10) *Mycobacterium abscessus massiliense, (35) Mycobacterium avium, (4) Mycobacterium chelonae, (12) Mycobacterium chimaera*, (2) *Mycobacterium furtuitum, (13) Mycobacterium xenopi, (4) Mycobacterium gordonae, (3) Mycobacterium kansasii*, *(11) Mycobacterium intracellulare* and (15) *Mycobacterium tuberculosis complex.* No differences were detected for strains cultivated in both solid and liquid cultures.

Discussion and Conclusions. The FTMA correctly identified 117 clinical isolates of NTM and 15 MTBC in 2.5 hours. Therefore, the FTMA could be a powerful improvement of the currently used diagnostic methods, reducing time to results and showing a high resolution compared to currently used techniques. Additional experiments will be needed in order to validate the instrument also directly from clinical samples.

107 - Hand hygiene and facemask use to prevent droplet transmitted viral diseases during air travel: a systematic literature review

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Background: Transmission of airborne viral diseases (e.g. influenza A H1N1) takes mainly place in confined spaces including public travel by aircrafts. This review summarizes the evidence on hand hygiene and use of facemask as viral disease prevention measures in aircrafts.

Methods: A literature search was performed in PubMed, Scopus, and Web of Science databases up to 10 June 2020, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria.

Results: We included four studies, all targeting the influenza virus disease, in the qualitative synthesis. Three studies used mathematical models, and two of them single flight models. The fourth was a case-control designed study to tracing an influenza outbreak in two flights during the 2009 influenza A H1N1 pandemic. Unlike the others, this study provided substantial evidence about the risk of not wearing a facemask in the airplane cabin during a flight from New York City to Hong Kong. Interestingly, none of 9 infected passengers compared to 15 (47%) of 32 healthy control passengers wore a mask (odds ratio, 0.0; 95% confidence interval, 0–0.7). In contrast, both case and control passengers appeared to be equally compliant in hand hygiene. Finally, we discussed the practicability of hygiene measures to control and prevent the SARS-CoV-2 transmission in confined air travel-related spaces.

Conclusions: Facemask use combined with other hygiene measures may minimize the chance of droplet-transmitted virus (including SARS-CoV-2) spread by air travelers. However, more evidence is necessary before hygiene measures become an integral part of standard procedures in aircrafts.

112 - Oropharyngeal cancers. and Human Papillomavirus in Southern Italy: histological findings and pathologic diagnosis. Preliminary data.

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Introduction
Only recently in Italy have been reported cases that correlate HPV and carcinomas of the head and neck district no data is currently available for Southern Italy in particular Sicily.
The aim of this study is to evaluate the possible oncogenic role of papillomavirus, on histological samples of squamous cell biopsies of the head and neck district diagnosed in the S. Elia hospital in Caltanissetta between 2015 and 2019.

Materials and methods
A retrospective study was conducted, based on the histopathological analysis of biopsy samples of carcinomas of the head and neck district divided into well differentiated (G1), moderately differentiated (G2) and poorly differentiated (G3) lesions. The role of HPV was analyzed by immunohistochemistry of the p16 expression pattern, considered a surrogate marker of the oncogenesis promoted by the papillomavirus.

Results.
We analyzed 26 hystopathological samples as follows: 8 of the oropharynx; 7 of the larynx and hypopharynx (respectively 6 and 1); 7 of the oral cavity; 4 of the nasopharynx. The age of patients is between 37 and 88 years with an average age of 65.3 years ± 11 (SD), 20 are male (76.9%) and 6 female (23, 1%). The youngest patients are those with nasopharyngeal carcinoma.Only lesions with a strong p16 expression pattern at immunohistochemistry were considered potentially related to papillomavirus, in total 7/26 (26.9%), of which 3 were oropharyngeal (37.5% of all oropharyngeal lesions), 1 of the oral cavity (14.2%), 2 of the larynx (28.5%) and 1 of the nasopharynx (25%). Of the HPV related lesions, 71.4% concern male subjects (2 oropharyngeal carcinomas, 1 oral cavity cancer, 1 laryngeal carcinoma and 1 nasopharynx carcinoma) and 28.6% female subjects (1 oropharyngeal carcinoma and 1 laryngeal carcinoma). All papillomavirus-associated carcinomas are moderately or poorly differentiated, in particular, 3 are moderately differentiated, 3 poorly differentiated and the nasopharyngeal carcinoma is undifferentiated. Therefore not considering nasopharyngeal carcinoma, as all the lesions observed are undifferentiated, 50% of the lesions are moderately differentiated and the remaining 50% are poorly differentiated. On the other hand, as regards related non-HPV carcinomas, always excluding those of the nasopharynx, 37.5% (6/16) of the carcinomas are well differentiated, 37.5% (6/16) moderately differentiated and 25% (4 / 16) poorly differentiated. As for nasopharyngeal carcinomas, these are all undifferentiated, including 1 related HPV. The statistical analysis conducted did not show a significant correlation between the expression of p16 and age, gender, location and grading.

Discussion.
Our study is among the few studies that reports an investigation on the possible correlation between head and neck cancers and HPV in our geographical area. The analysis confirmed, as demonstrated by other studies in the literature, the presence of some potentially HPV squamous carcinomas of the head and neck district and also highlighted different expression patterns for p16 of unclear meaning.

141 - Whole genome sequence of the SARS-CoV-2 Siena-1/2020 viral isolate

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Introduction

The severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) pandemic has caused more than 800.000 deaths globally and more than 35.000 deaths in Italy. Sequencing of viral isolates and direct sequencing from clinical samples are useful tools for genetic epidemiology. Here we present the sequence of the first viral strain isolated in Tuscany.

Materials and Methods

The SARS-CoV-2 Siena-1/2020 isolate was obtained by seeding a nasopharyngeal swab sample on Vero E6 cells. Total RNA was extracted from culture medium collected after the appearance of cytopathic effect. The complete genome was obtained by Nanopore sequencing combining direct RNA sequencing and amplicon sequencing approaches.

Results

We sequenced 29.901 bases of the Siena-1/2020 isolate with a mean depth of coverage of 1153.64. The GC content was 37,97%. Comparison with the reference genome Wuhan Wu-1 showed that Siena-1/2020 harbors 5 single nucleotide changes (at positions 241, 3037, 14408, 19839, and 23403) and mutation of three consecutive nucleotides (GGG→AAC) at position 28881. Among the 5 single nucleotide changes, the one at position 23403 causes a change in the predicted amino acid sequence of the S (spike) protein (D614G), which is now the most common variant worldwide. Phylogenetic analysis performed with Pangolin (v1.14) assigned the Siena-1/2020 strain to the B.1.1 lineage which is associated with the Italian SARS-CoV-2 outbreak and includes isolates circulating in Europe.

Discussion and Conclusions

We obtained the genome sequence of the first SARS-CoV-2 viral isolate from Tuscany. We combined direct RNA and amplicon sequencing approaches, both performed with Nanopore technology. We developed a new primer scheme for amplicon sequencing which can also be applied to RNA from clinical samples to rapidly obtain complete or near complete genome sequences for epidemiological studies.

6 BIOFILM

6 - Characterization of Scardovia wiggsiae biofilm by scanning electron microscopy

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Introduction: Recently a newly identified anaerobic *Bifidobacterium* named *Scardovia wiggsiae* has been related to the Early Childhood Caries (ECC), a particularly severe manifestation of carious pathology with rapid and disruptive progression, whose microbiota includes a wide variety of bacterial species. This study aims at providing the first ultrastructural morphological characterization of *S. wiggsiae* and its biofilm, adopting an innovative protocol for scanning electron microscopy (SEM).

Materials/methods: *S. wiggsiae* DSM 22547 strain was cultivated at 35°C for 48h in anaerobic chamber, transferred in tubes containing discs of bioglass (a material that mimics dentin surface) and incubated for 120 h to allow biofilm growth. *S. wiggsiae* was investigated for its ability to produce biofilm on polystyrene plate, and biofilm was quantified by crystal violet staining. *S. wiggsiae* biofilm on bioglass discs was observed at SEM (VPSEM Hitachi SU 3500) after fixation with glutaraldehyde and treatment with a mixture of osmium tetroxide and Ruthenium Red. Samples were mounted on aluminum stubs and dried in a desiccator at 40 °C.

Results: Based on quantification of the total biomass at 590 nm, *S. wiggsiae* can be considered a strong biofilm producer. At low magnifications (1000X) in SEM we saw a well preserved biofilm aspect including areas with different surface texture. Very high magnifications (ranging from 10000X to 35000X) allowed to identify bacterial cells embedded in the matrix bulk. Focusing on matrix trabeculae, in both spongy and granular areas, it was possible to distinguish an heterogeneous density. Biofilm matrix surface was irregularly dotted with small globular aggregates. At extremely high magnifications also the surface appeared irregular due to the presence of very fine globular aggregates of matrix components. Where matrix was less dense, numerous and intimately packed bacteria were visible. In these areas the ultrastructural morphology of the individual bacterial cells could be appreciated. Cell length and diameter were measured.

Discussion and Conclusions: We report the first ultrastructural description of *S. wiggsiae* biofilm and the morphology of the bacterial cells embedded in biofilm. To this aim an innovative and unusual protocol for SEM analysis was adopted.

10 - Material characterization and Streptococcus oralis adhesion on Polyetheretherketone (PEEK) and titanium surfaces used in implant dentistry.

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Introduction. Polyetheretherketone, PEEK, is a thermoplastic polyaromatic semi crystalline polymer, recently introduced in implantology. The aim of this work was to verify if PEEK interaction with *Streptococcus oralis* could be more performant on bacterial adhesion inhibition in respect to two titanium surfaces used in implantology: machined and double acid etched (DAE). The secondary outcome was the material characterization of the three types of samples, in order to increase the knowledge of the parameters that could affect the bacterial colonization. A correlation between the superficial nano- and micro- structure of the three discs and a microbiological analysis was performed.

Materials and Methods. The superficial micro-roughness of the PEEK discs was analyzed by scanning electron microscopy (SEM) and the Energy Dispersive Spectrometer (EDS) analyzed their chemical composition. Atomic Force Microscopy (AFM) was used to characterize the micro-topography and the sessile method to evaluate the wettability of the samples. Microbiological analysis measured the colony forming units (CFUs) for the quantification of cultivable cells, the biofilm biomass by Hucker's crystal violet staining method and the cell viability by Live/Dead analysis after 24 and 48 h of *S. oralis* clinical strain cultivation on the different discs, previously incubated with saliva**.**

Results. The SEM observations showed that PEEK was characterized by a micro-roughness similar to machined titanium but at AFM images the nano-roughness was significantly higher in respect to the other samples. The EDS analysis showed that PEEK samples were mainly composed by Carbonium and Oxygen.The hydrophilicity and wetting properties of PEEK were similar to machined titanium; on the contrary, double etched discs (DAE) samples were characterized by significantly higher levels ($p<0.05$). Microbiological analysis demonstrated that PEEK was characterized by significant lower CFUs, biofilm biomass formation and viable cells in respect to the titanium surfaces. No differences were found between machined and DAE discs.

Discussion and Conclusions. In the complex, both wettability and nano-roughness of PEEK are able to significantly affect the CFUs and biofilm biomass formation.The results of this study underlined the bactericidal and/or anti-adhesive action of PEEK surfaces, pre-treated by saliva mimicking the oral cavity environment against an early colonizer such as a clinical isolate of *S. oralis*. The anti-adhesive and antibacterial properties showed by PEEK at 24 and 48 h against a pioneer such as *S. oralis*, could have an important role in the prevention of all pathologies connected with biofilm formation, like peri-implantitis in dentistry or prosthetic failures in orthopaedics.

22 - Graphene Oxide affects S. aureus and P. aeruginosa Lubbock Chronic Wound Biofilm model

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Introduction. Chronic wounds management is a complex procedure due to the persistence of pathogens forming biofilm that is a key virulence determinant in the wound chronicization. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the prevailing bacterial species that co-infect chronic wounds resulting more refractory to the treatment because of the biofilm structural integrity. Aim of this paper is to detect the Graphene Oxide (GO) effect on *S. aureus* and *P. aeruginosa* dual species wound biofilm in Lubbock Chronic Wound Biofilm (LCWB) model. LCWB model is the first *in vitro* model that simulate a realistic *in vivo* multispecies biofilm that develops into chronic wounds allowing the biofilm growth of a multispecies bacterial population.

Materials and methods. Clinical isolates *S. aureus* PECHA 10 and *P. aeruginosa* PECHA 4 are the pathogens used in this study. The GO effect on in forming and mature dual species biofilms is detected by the evaluation of the CFU/mg reduction in respect to the controls, the cell viability and ultrastructural analysis of the treated LCWBs.

Results. Graphene Oxide, at 50 mg/l, is capable to inhibit the growth of clinical isolates from chronic wounds in LCWB model. In particular, during the biofilm formation, GO reduces the *S. aureus* and *P. aeruginosa* growth of 55.05% ± 4.73 and 44.18% ± 3.91 compared to the control. In mature biofilm, GO affects *S. aureus* and *P. aeruginosa* by reducing their growth of 70.24% ± 4.47 and 59.31% ± 16.84, respectively. Images taken by Scanning Electron Microscopy (SEM) show that GO, together with a disaggregated microbial effect, expresses its action also disrupting the fibrin network.

Discussion and Conclusions. Graphene Oxide reduces the *S. aureus* and *P. aeruginosa* dual species biofilm in LCWB model and inhibits a proper polymerization of fibrin during biofilm matrix formation. In conclusion, the GO use against microorganisms grown in LCWB, displays a significant inhibitory action resulting in a promising tool for potential application in wound management. Graphene Oxide can be used to improve the wound healing, enhancing the rate of wound repair and reducing the scar formation.

24 - Evaluation of benzydamine effects on Candida albicans adhesion, biofilm formation and persistence onto abiotic surfaces.

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Introduction. *Candida albicans* is the most abundant yeast colonizing the oral cavity. It behaves as an opportunistic pathogen, causing mucosal infections mainly in immunocompromised individuals; in addition, it is often associated to patients suffering from diabetes, oral cancer and terminally ill conditions. Benzydamine hydrochloride is a non-steroidal and anti-inflammatory agent. It has been included in the formulation of several mouthwashes because endowed with analgesic and anesthetic properties. Since benzydamine exerts antibacterial and antifungal activity *in vitro*, we assessed if this molecule could affect *C. albicans* virulence traits, such as adhesion, biofilm formation and persistence on abiotic surfaces.

Materials and Methods. *C. albicans* CA1398, carrying the bioluminescence ACT1p-gLUC59 fusion product, was employed. Firstly, fungal cells were exposed for 1', 5', or 15' to 4 different benzydamine concentrations (0.075%, 0.15%, 0.3% and 0.6%) and then tested for their capacity to adhere to plastic (90' incubation) or to form a biofilm (24h assay). Secondly, 24 and 48h-old biofilms were exposed to the same concentrations of benzydamine and for the same times in order to assess biofilm persistence and regrowth. Benzydamine effects were quantified by measuring, in parallel, metabolically active fungal cells (bioluminescence assay) and viable cells (Colony Forming Units assay).

Results. Benzydamine impaired ability to adhere to plastic and to form biofilm, in a dose-dependent fashion; such effects could be ascribed to a direct effect of benzydamine on Candida viability only when using the highest dosage. Moreover, benzydamine caused a dose-dependent decrement in the viability of Candida cells embedded in biofilm, no matter whether a 24h- or a 48h-old sessile community was tested.

Discussion and Conclusions. Benzydamine not only impairs *C. albicans* biofilm formation, profoundly affecting the initial step of fungal cell adhesion to abiotic surfaces, but it is also able to counteract persistence and regrowth of a preformed biofilm. The capacity of benzydamine to affect *C. albicans*, a fungus responsible of oral diseases in several categories of susceptible subjects, makes this molecule a very interesting tool for both prevention and treatment of oral candidiasis. Studies employing benzydamine-containing mouthwashes will be carried out, in order to assess and compare the anti-Candida effects of different commercial products.

28 - Antibiofilm and antivirulence activity of glucocorticoid PYED-1 against Stenotrophomonas maltophilia

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Introduction
Stenotrophomonas maltophilia, an environmental Gram-negative bacterium, is an emerging nosocomial opportunistic pathogen that causes life-threatening infections in immunocompromised patients and chronic pulmonary infections in cystic fibrosis patients. Due to increasing resistance to multiple classes of antibiotics and the ability of this bacterium to form highly structured and multilayered biofilms, *S. maltophilia* infections are difficult to treat successfully. This makes the search for new antimicrobial strategies mandatory. The aim of this study was to evaluate the antimicrobial, antibiofilm, and antivirulence activities of steroid derivatives against *S. maltophilia*.

Materials and Methods
The MIC values of deflazacort (DFZ), and its precursors against planktonic bacteria were measured by broth microdilution method, while the activity of steroid derivatives against bacterial biofilms was tested by crystal violet and tetrazolium salt reduction assay. The combination effects between PYED-1 and gentamicin or amikacin against *S. maltophilia* K279a cells were assessed by a microbroth checkerboard assay. The transcription of selected virulence-related genes was verified by quantitative reverse transcription real-time polymerase chain reaction (RT-qPCR) analysis.

Results
The antibacterial activity of the heterocyclic corticosteroid DFZ and six of its precursors was tested against *S. maltophilia*. All compounds were not active against standard strain *S. maltophilia* K279a. The compound PYED-1 (pregnadiene-11-hydroxy-16alpha,17alpha-epoxy-3,20-dione-1) showed a weak effect against some *S. maltophilia* clinical isolates but exhibited a synergistic effect with aminoglycosides. PYED-1 at sub-inhibitory concentrations decreased *S. maltophilia* biofilm formation. A promising alternative strategy to treat infections caused by multidrug bacteria is the development of drugs able to specifically inhibit virulence factors. To investigate the antivirulence activity of PYED-1 the expression levels of *S. maltophilia* virulence genes were investigated by RT-qPCR. This analysis demonstrated that the expression of biofilm- and virulence- associated genes (*stmPr1*, *stmPr3*, *sphB*, *smeZ*, *bfmA*, *fsnR*) was significantly suppressed after PYED-1 treatment. Interestingly, PYED-1 also repressed the expression of the genes *aph (3´)-IIc*, *aac (6´)-Iz*, and *smeZ*, involved in the resistance to aminoglycosides.

Discussion and Conclusion
PYED-1 in combination with gentamicin or amikacin aminoglycosides represent a significant tool to control *S. maltophilia* growth. Moreover, PYED-1 was identified as a promising agent for targeting biofilm and virulence of *S. maltophilia*. This might be a new strategy for the treatment of *S. maltophilia* biofilm-associated chronic infections.

51 - An in vitro biological and anti-bacterial study on 3D printed multi-layer silver-incorporated biomaterial.

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Introduction: Calcium phosphates (CPs) scaffolds for bone tissue engineering require a combination of biological properties, such as osteoconductivity and osteoinductivity, and sufficient mechanical strength to match the host bony tissue. In addition, the improvement of their antimicrobial properties to prevent microbial adhesion by incorporation of heavy metals, such as silver, is really necessary. For these reasons, new functionally-graded materials can be designed, in which variations of structural and functional properties lead to the desired multifunctionalities. In this framework, a CP-based multilayer scaffold was designed and developed with the aim of providing mechanical, bioactivity and antibacterial properties at the same time.

Materials and methods: The multilayer scaffolds were developed by additive manufacturing with a ceramic bilayer structure made of a dense CP core and an external CP macro-porous layer, printed in a single step and a biphasic CP, made of a mixture of hydroxyapatite and □-tricalcium phosphate. Then, a porous polycaprolactone (PCL)/CP composite layer was cast around the sintered bilayer samples, in which the PCL layer was further modified with Ag ions, to provide antibacterial properties. The bioactivity properties of the produced samples were tested *in vitro* by soaking the sintered samples in simulated body fluid. Antibacterial tests were performed by assaying *Staphylococcus aureus* adhesion on biomaterials through a sonication protocol to dislodge adherent microorganisms without altering their viability. The planktonic bacteria number was also determined.

Results: Homogeneous bilayer ceramic scaffolds were successfully produced by the robocasting technique, in which the porous part was well connected with the inner ceramic core, which showed a compressive strength higher than 50 MPa. FESEM observations revealed that these ceramic samples were covered by an apatite layer confirming their osteoconductivity. The polymeric layer well adhered to the ceramic structure, and the antibacterial tests revealed a significant ($p<0.001$) reduction of the adherent staphylococci on the Ag-functionalized surfaces, after 24 h of incubation, with values of about 10^4 CFU/ml with respect to 10^9 CFU/ml for controls. Additionally, a similar significant ($p<0.001$) decrease in CFU/ml was detected for planktonic bacteria, thus proving the Ag release from the enriched PCL-based samples.

Discussion and conclusions: Given the competition between tissue integration and microbial colonization inherent in the race for the surface, a frequently asked question is whether new tissue-engineered scaffolds will provide new opportunities to win the race. The robocasting biomaterial here designed, exhibited a complex architecture capable to determine osteoinduction and an ideal antimicrobial strategy against surface-adherent biofilm bacteria, but also against bacteria in the tissue surrounding the biomaterial.

52 - Synergic Antifbiofilm activity of the human lactoferricin derived peptide hLF1-11 in combination with caspofungin against Candida albicans and Candida parapsilosis

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Introduction. Candida species are the main fungal opportunistic pathogens causing mucosal and systemic infections often associated with drug resistance and biofilm production on medical device. Biofilm associated infections are difficult to treat and when evolve in candidaemia, are often associated with an increase of morbidity and mortality. The difficulty to find new antifungal drugs has led to an increased interest in the use of antimicrobial peptides, alone or in combination with conventional drugs. *The present study was aimed at investigating the possible synergistic activity of a synthetic peptide* hLF1–11 and caspofungin *against Candida albicans* and *Candida parapsilosis* biofilm in vitro.

Materials and Methods. The interaction between caspofungin and hLF1–11 was evaluated by the checkerboard assay using 96-well flat bottom polystyrene microtiter plates. Yeast cells (*10^6 CFU/mL) were incubated with the antifungal compounds, alone or in combination, for 24 h at 37°C.* The synergistic activity was evaluated for *C. albicans* on a reference strain (SC5314) and a caspofungin resistant (MIC:2 µg/mL) clinical isolate (CACR), and for *C. parapsilosis* on a reference strain (ATCC 22019) and a clinical isolate (CP7) selected for its strong ability to produce biofilm.
Synergism was evaluated by XTT reduction metabolic assay in the checkerboard plate and then, reduction of cell viability was evaluated in selected concentrations with low metabolic activity.

Results. A strong inhibitory synergistic effect in biofilm production was observed against all the tested *C. albicans* and *C. parapsilosis* strains. A fractional inhibitory concentration (FIC) index ≤0.5 was interpreted as synergy, as a decrease in CFU/ml of ≥2 Log by the combination of hLF1–11 and caspofungin in comparison with the most active constituent. Synergistic effect was obtained with 32-fold MIC reduction compared to the MIC of hLF 1-11 alone and with 16-fold MIC reduction compared to MIC of caspofungin alone.

Discussion and Conclusions. The synergistic effect observed between caspofungin and hLF1-11 against *C. albicans* and *C. parapsilosis* biofilm production represents an interesting novel approach to target drug resistant fungal biofilm infections.
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59 - The antibiofilm activity of Grifola frondosa extracts

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Introduction: Albino *Grifola frondosa*, also called Maitake, is a wild edible mushroom native to northeastern Japan also distributed in Europe, Asia and in the eastern belt of the North American continent. It is acknowledged for its nutrional and medical properties as antitumor, antioxidant and antimicrobial activity in vitro and anti-aging in vivo. For these reasons, it is valuable for the pharmaceutical industry. Therefore, the aim of the study was to test the antibiofilm effect of mushroom extracts on strains of *Staphylococcus aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 that are microorganisms widely involved in nosocomial infections. These bacteria are increasingly resistant to drugs and the resistance is made more effective also thanks to their ability to form biofilm. Indeed, it is very useful to identify substances that can eliminate them or block their proliferation and that do not harm patients.

Materials and Methods: The bacterial strains were inoculated in Trypticas Soy Agar media and incubated at 37°C for 24 h. The bacterial suspensions (0.5 McFarland in Broth Tryptose, BT), more precisely 100 µL of overnight grown culture, were added in a sterile 96-well flat bottom microtiter. For whites, only the BT medium was dispensed. To evaluate biofilm production, excess bacterial suspension was removed. Subsequently, the mushroom extracts (25% v/v) were re-suspended in BT, 100 µL were added in the wells and the cultures were incubated at 37 ° C for 24h. The biofilm was colored with crystal violet dissolved in ethanol (0.5% w/v). The optical density was measured at 540 nm.

Results: The mushroom extracts decreased the biofilm produced by *S. aureus* ATCC 43300 whereas it slightly enhanced biofilm formation by *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853.

Discussion and Conclusions: The results obtained showed a good efficacy in relation to the antibiofilm action of the Albino *G. frondosa* extracts against the ATCC strains of *S. aureus*. Considering the total edibility of the investigated mushroom from which the extracts were obtained, their effect could be enhanced with the increasing of the concentration. It is planned to evaluate the action of these extracts in the future also on some strains which play an important role in nosocomial infections.

65 - Antibacterial secondary metabolites from pathogenic fungi of forest trees. Anti-biofilm activity of the pimarane diterpene sphaeropsidin A against clinical isolates of antibiotic-resistant bacteria.

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Introduction: Many pathogens involved in human infection rapidly increased their antibiotic resistance reducing the effectiveness of therapies in recent decades. Most of them can form biofilms and effective drugs are unavailable to treat these formations. Natural products could represent an efficient solution to overcome the antimicrobial resistance and treat biofilm-related infections. In this study, 20 secondary metabolites produced by pathogenic fungi of forest plants were evaluated for the first time against antibiotic-resistant staphylococci and *P. aeruginosa.*

Materials and methods: The initial screening was performed by broth micro-dilution assay to test a single high concentration against all test strains. Subsequently we determined Minimal Inhibitory and Bactericidal Concentrations (MIC and MBC) of selected compounds and their ability to prevent biofilm formation using crystal violet staining. The interactions between the selected metabolites were also evaluated by the checkerboard method. Cytotoxicity tests were performed on HaCaT cells.

Results: The cyclohexene oxides *epi*-epoformin and sphaeropsidone, and the pimarane diterpenoid sphaeropsidin A showed activity on all test strains. Particularly, sphaeropsidin A was effective at low concentrations with MIC values ranging from 6.25 μg/mL to 12.5 μg/mL. Mixtures of sphaeropsidin A and *epi*-epoformin have shown antimicrobial synergistic effects with a concomitant reduction of cytotoxicity against HaCaT cells. Furthermore, sphaeropsidin A at sub-inhibitory concentrations decreased MRSA and *P. aeruginosa* biofilm formation.

Discussion and conclusions: The screening performed showed interesting activities for *epi*-epoformin and sphaeropsidin A against antibiotic-resistant staphylococci and *P. aeruginosa* and can represent a promising basis to identify scaffolds with antimicrobial potential. *epi*- Epoformin and sphaeropsidin A contain structural features known to be responsible for such activity in naturally occurring compounds. These functional groups are the epoxy group (in *epi*-epoformin) and the alpha-, beta-unsaturated ketone group (in *epi*-epoformin and sphaeropsidin A), which could react with a nucleophilic group of the receptor (such as -NH2 or -SH, etc.). Our results represent preliminary data on the antimicrobial activity of fungal secondary metabolites evaluated for the first time against common human pathogens. The characterization of the action mechanism of these compounds and the introduction of chemical modifications in their molecules could lead to the synthesis of derivatives with acceptable biocompatibility and improved antimicrobial properties against multi-resistant and biofilm-producing bacteria.

103 - Effects of a polycationic choline-calix[4]arene amphiphile on growth, biofilm and motility of Escherichia coli and Pseudomonas aeruginosa

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Introduction. The high incidence of drug-resistance and biofilm associated infections is still a major cause of morbidity and mortality and triggers the need for new antimicrobial drugs. Calix[n]arenes, a family of macrocyclic compounds formed by a number [n] of phenolic unities linked by methylene bridges, are largely studied as molecular scaffolds in antibiotic discovery. The introduction of multiple bioactive groups in the calixarene macrocycle can provide innovative multivalent compounds against which resistance mechanisms have not yet been developed. Amphiphilic calixarene derivatives can also generate nano-antibiotics that might fill the gaps where traditional antibiotics fail. By exploiting their physical as well as chemical properties, nano-antibiotics can use alternative mechanisms of action. In this study, a polycationic choline-calix[4]arene amphiphile (Chol-Calix) bearing hydrophilic choline moieties and hydrophobic dodecyl aliphatic chains linked at the four calix[4]arene phenolic rings, was evaluated for its inhibitory effects against *Escherichia coli* and *Pseudomonas aeruginosa* growth, biofilm and motility.

Materials and Methods. The susceptibility of *E. coli* ATCC 10536 and *P. aeruginosa* ATCC 9027 to Chol-Calix was performed by the microdilution assay. The effect of Chol-Calix on biofilm formation and preformed biofilm was assessed in microtiter plates by measuring the total biomass and by observing the biofilm under the light microscope. After exposure to sub-inhibitory concentrations (sub-MICs) of Chol-Calix, the swarming, swimming and twitching motility was measured as the diameter of zone travelled by bacteria point- or stab-inoculated into semi-solid media.

Results. Chol-Calix inhibited effectively the growth of *E. coli* and *P. aeruginosa* with MIC values of 18.8 - 9.4 mg/L, respectively. Chol-Calix resulted effective against biofilms causing the inhibition of biofilm formation (60-80%) at subMICs and promoting the degradation of mature biofilm (40-70%) at concentrations above the MIC. The microscopic images revealed a reduced biofilm with few cells. Motility tests demonstrated that Chol-Calix decreased the cell motility in both *E. coli* and *P. aeruginosa*.

Discussion and Conclusions. Our results clearly evidenced the antibacterial properties of Chol-Calix. It is plausible to hypothesize that the antibacterial and antibiofilm activities and motility inhibition of the polycationic Chol-Calix are related to its ability to establish electrostatic interactions, intermolecular hydrogen bonding and hydrophobic interactions with molecular components of the bacterial membrane and biofilm. Antibacterial properties, nanosized structure and known low cytotoxicity on eukaryotic cells make Chol-Calix a promising new antibiotic candidate.

136 - Activity of Satureja montana essential oil structured in nanoemulsions on biofilm production by avian Escherichia coli strains

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Introduction It is known that poultry meat exhibits the highest overall levels of *E. coli* contamination. *E. coli* ability to form microbial communities, such as biofilm, contributes to the bacterial persistence on surfaces of poultry products. Since antimicrobial resistance in farm animals represents a serious problem, commensal *Escherichia coli* living in the gut of animals has typically been selected as antimicrobial resistance sentinel. Recently, several Authors reported that essential oils (EOs) from different species of *Satureja*, belonging to the botanical family of *Lamiaceae*, possess remarkable antibacterial activity. Nanoemulsions (NEs), colloidal dispersion in which main components are oil, an emulsifying agent and aqueous phase, are able to improve the EOs properties. In our study the antibiofilm activity of new designed Oil in Water NEs, composed by SEO (*Satureja* Essential Oil) and Tween-80 was assayed.

Materials and Methods Avian *E. coli* strains were obtained from a laboratory collection of characterized bacterial isolates. NEs were produced by a ratio of 1:1 with SEO and Tween-80 and characterized for size and stability. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for EOs and NEs was estimated by the broth micro-dilution method. Quantification and eradication of biofilm production in the presence of SEO and NEs was assessed by crystal violet staining. The cytotoxicity of free SEO and structured in NEs was determined on human T-24 bladder cells by MTT assay.

Results Results showed that 30% and 27% of *E. coli* strains possessed, respectively, a strong and moderate ability to form biofilms; 23% were weakly biofilm strains producers and 20% was totally unable to produce biofilm. MIC/MBC values of SEO and NEs ranged from 0.78 to 3.12 mg/mL. Interestingly, while SEO was bactericidal for 43% of the strains, SEO in NEs was for 100%. Sub-MIC concentrations of SEO, free or in NEs, significantly reduced biofilm production of the strong producer strains, whereas they were unable to efficiently eradicate the preformed biofilm. The survival of cells exposed to NEs was significantly higher than that found in samples treated with the same amount of free oil.

Discussion and Conclusions Recently some studies suggest that bioactive compounds present in EOs, such as eugenol and thymol, could reduce the formation of biofilm in *E. coli*. Our results about bactericidal activity of SEO loaded in NEs suggest a potential use of this system as sanitizing and disinfection treatment in farm animal, in alternative to antibiotic management that could encourage the selection of resistant microorganisms. Moreover, the use of EOs structured in NE could represent also a new frontier in terms of safety and efficacy.

7 PATOGENICITA' MICROBICA

1 - The beta-lactamase inhibitor boronic acid SM23 inhibits Pseudomonas aeruginosa biofilm formation and virulence factor production

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Introduction: *Pseudomonas aeruginosa* is a Gram-negative nosocomial pathogen, often responsible of severe device-related infections, given its great ability to produce biofilm. *P. aeruginosa* finely regulates the expression of different virulence factors, including biofilm production, by Quorum Sensing (QS), an intercellular communication mechanism used by many bacteria. Biofilm formation enhances bacterial resistance to antimicrobial agents due to a decreased penetration of antibiotics and a reduced rate of growth of embedded bacteria. Thus, novel agents capable of selective inhibiting biofilm formation may represent a promising strategy to overcome the well-known and widespread drug-resistance of *P. aeruginosa*.

Material and Methods: by using the bioluminescent *P. aeruginosa* strain P1242, we investigated the effects of SM23, a boronic acid derivative specifically designed as beta-lactamase inhibitor, on biofilm formation and virulence factor production by *P. aeruginosa*.

Results: we found that SM23: a) inhibited both biofilm formation and production of the virulence factors, pyoverdine, elastase and pyocyanin, without affecting bacterial growth; b) decreased the levels of QS-related autoinducers molecules, namely *3-oxo-C_{12}-HSL* and *C_4-HSL*, by reducing *lasR*/*lasI* system gene expression in the biofilm; c) failed to bind to bacterial cells that had been preincubated with *P. aeruginosa*-conditioned medium; d) reduced significantly *P. aeruginosa* biofilm and pyoverdine production on endotracheal tubes, an *in vitro* condition closely mimicking clinical settings.

Discussion and Conclusions: taken together, our results indicate that, besides inhibiting beta-lactamase, the boronic acid SM23, can also act as potent inhibitor of *P. aeruginosa* virulence, by profoundly affecting biofilm and QS-related signals. These findings highlight potential application of this compound in the prevention and treatment of biofilm-associated *P. aeruginosa* infections.

5 - New SARS-CoV-2 infection in a Pet Cat with severe lung disease in Italy

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Introduction: the pandemic respiratory disease COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan in December 2019 and then spread throughout the world; Italy was the most affected European country. Despite the close pet-human contact, little is known about the predisposition of pets to SARS-CoV-2. Among these, felines are the most susceptible.

Materials and Methods: in this study, a nasal swab of a pet cat with clear symptoms of pneumonia, confirmed by Rx imaging, was analyzed by using quantitative RT–qPCR and subsequent gene sequencing.

Results: RT-qPCR amplification was positive for two different SARS-CoV-2 genes and sequencing confirmed the identity of the human SARS-CoV-2.

Discussion and Conclusions: this is the first Italian study reporting on the request of the scientific community to focus attention on the possible role of pets as a SARS-CoV-2 reservoir. An important question remains unanswered: did the cat die from SARS-CoV-2 infection?

8 - Propolis extracts characterization and evaluation of their efficacy against Pseudomonas aeruginosa and its virulence factors

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1. Introduction: *Pseudomonas aeruginosa* (*P. aeruginosa*) is a Gram-negative opportunistic pathogen, responsible of wide spectrum infections, especially in hospitalized individuals. Its clinical relevance is linked to the numerous virulence factors, including the ability to produce biofilm and the release of key molecules, such as quorum sensing signals, phenazines, eDNA, etc. By an engineered bioluminescent (BLI)-*P. aeruginosa* strain, our *in vitro* study was aimed at characterizing different propolis extracts and assessing their effects on *Pseudomonas* and its virulence factors.

2. Materials and Methods: Imprimis, crude extracts of Albanian propolis were prepared using dynamic maceration with three solvents, namely absolute ethanol (EtOH), propylene glycol (PG) and polyethylene glycol 400 (PEG 400). The three propolis extracts were tested for: a) polyphenolic compounds content through High Performance Liquid Chromatography–Mass Spectrometric (HPLC-MS) analysis, b) anti-*P. aeruginosa* activity by Minimal Inhibitory Concentration (MIC) assay, c) anti-microbial growth and anti-biofilm effects, kinetically evaluated by bioluminescence assay and d) effects on phenazines production and eDNA release, assessed by HPLC-ESI-MS and fluorescence assay, respectively.

3. Results: The three extracts exhibited similar profiles in terms of polyphenols content. The MIC values of propolis EtOH and PG extracts were superimposable (15.6 μg/mL), while the MIC of propolis PEG 400 extract was as high as 62.5 μg/mL. EtOH extract was the most effective in inhibiting both microbial growth and biofilm formation, followed by PG and then PEG 400 propolis extracts. Such inhibitory effects were associated with a relevant impairment in eDNA release; also, phenazines production was deeply affected. Finally, upon propolis EtOH extract exposure to *Pseudomonas*, a relevant consumption of caffeic acid phenethyl ester (CAPE) and quercetin was detected.

4. Discussion and Conclusions: Antimicrobial activity of propolis is not as good against Gram-negative bacteria, likely because of their peculiar structure and their ability of producing a wide range of hydrolytic enzymes, which in turn may break down the active compounds of propolis. Here, we show that, among three different extracts, EtOH-propolis has the best performance in inhibiting all the tested *Pseudomonas*-associated virulence factors. Concomitantly, propolis exposure to the pathogen allows an interesting consumption of relevant antimicrobial compounds, such as CAPE and quercetin. By this *in vitro* study, we provide novel insights on the anti-*P. aeruginosa* properties of propolis and the compounds possibly involved.

Keywords: propolis, polyphenols, CAPE, quercetin, *P. aeruginosa*, bioluminescence, phenazines, eDNA, MIC

12 - Evaluation of in vitro synergy of honey, myrtle and pomegranate polyphenolic extracts against oral pathogens

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Introduction: The increasing incidence of oral diseases, the wide spread of antimicrobial resistance and the adverse effects of the conventional antibiotics, needs alternative prevention and treatment options to counteract oral pathogens. In this regard, this study evaluates the antibacterial activity of polyphenolic extracts from acacia honey, myrtle leaves and pomegranate peel against cariogenic bacteria as *Streptococcus mutans* and *Rothia dentocariosa*.

Materials and Methods: All the extracts were used singly and in binary combinations to highlight any synergistic effects. Moreover, the extracts were also tested in association with amoxicillin to evaluate their ability to reduce the effective dose of this drug *in vitro*. The values of minimal inhibitory concentrations and minimal bactericidal concentrations were used to quantitatively measure the antibacterial activity of the single extracts, while the fractional inhibitory concentration index was considered as predictor of *in vitro* anticariogenic synergistic effects. Time-kill curve method allowed to evaluate the bactericidal efficacy of combined extracts.

Results: Total polyphenol content in honey, myrtle and pomegranate extracts, indicates as mg of gallic acid equivalents (GAE) for g of sample, was 15,75 mg GAE g^{-1} for acacia honey extracts, 54,86 mg GAE g^{-1} for myrtle leaves extracts and 45,9 mg GAE g^{-1} for pomegranate peel extracts. The chemical-physical parameters of acacia honey and the pomegranate peel extracts RP-HPLC polyphenolic profile have been previously described. The myrtle extracts characterization, performed by HPLC analysis showed that the most abundant components were Galloyl derivatives and tannins as phenolic acids and Myricetin and Quercetin derivatives as flavonols. Hydro-alcoholic polyphenolic extracts from pomegranate, myrtle and honey showed bacteriostatic and bactericidal effects against *S. mutans* and *R. dentocariosa* strains. The inhibitory effect of extracts was evident when used individually and in binary combination. In particular, the combination of pomegranate peel and myrtle leaves polyphenolic extracts, compromised the survival of both bacterial isolates in a dose-related manner with bacteriostatic and bactericidal effects both against pure bacterial cultures and against mixed bacterial cultures of *S. mutans* and *R. dentocariosa*.

Discussion and Conclusions: Results suggest that acacia honey, myrtle and pomegranate extracts inhibit cariogenic bacteria, even with synergistic effects and encourages the use of natural extract combinations alone or in association with antibiotic as a valid alternative for the prevention and treatment of oral infectious diseases.

20 - Reduction of Polyomavirus microRNA-5p expression in saliva after short-term of kidney transplantation as indicative factor for viral reactivation

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Background. Polyomavirus (PyV) microRNAs present in several biological fluids are suggested relevant viral expressing factors to monitoring PyV persistence. Since infections caused by persistent viruses such the eight human herpesviruses and polyomavirus continue to challenge the clinical management of transplant recipients, it is of relevance to monitor their reactivation in transplanted patients.

Methods. Here it was investigated the PyV-microRNA-5p expression of JCPyV, BKPyV, MCPyV and SV40 in paired saliva and plasma samples obtained from 23 patients before and after short-term renal-transplantation. PyV-microRNA-5p expression was analysed and quantified with the specific JCPyV (jcv-miR-J1-5p), BKPyV (bkv-miR-B1-5p), MCPyV (mcv-miR-M1-5p) and SV40 (sv40-miR-S1-5p) miRNA-5p quantitative stem-loop RT-PCR MiRNA assay.

Results. Overall patients at short-time after transplantation exhibited significantly decreased of leukocytes and lymphocytes cells, creatinine amount but no substantial herpes virus reactivation during their clinical management. PyVs-microRNA-5p in saliva samples reported a significant high number of positive samples (range 22%-91%) to three polyomavirus microRNA-5p (JCPyV, BKPyV and SV40) compared to those (range 9%-35%) observed in plasma paired samples. Among the three polyomavirus microRNA positive observed, BKPyV-microRNA-5p exerted the highest positivity (91%). Moreover, saliva samples exhibited alsoa quite constant high polyomavirus microRNA copies number compared to that of the paired plasma samples. Additionally, 23 and 16 saliva samples and 7 and 8 plasma samples were positive at least for one PyV-microRNA-5p, before and after transplantation, respectively. Of note, compared to time before, in saliva samples obtained after transplantation significant reduction of positive status for total PyVs-microRNA-5p and for 2 out of 3 single PyVs-microRNA-5p was reported. Additionally, at time post transplantation it was observed inverse correlation with the JCPyV-, BKPyV- and SV40- microRNA-5p expression in saliva with the serum creatinine concentration.

Conclusion. Collectively, these data suggest that investigations of PyV-microRNA-5p present in saliva at short-time of renal-transplantation may be useful to shed light on thePyV early event implicated in the potential viral reactivation.

31 - D-mannose treatment neither affects uropathogenic Escherichia coli properties nor induces stable FimH modifications

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Introduction. Urinary tract infections (UTIs) are mainly caused by uropathogenic *Escherichia coli* (UPEC). Acute and recurrent UTIs are commonly treated with antibiotics, the efficacy of which is limited by the emergence of antibiotic resistant strains. The sugar D-mannose is considered as an alternative to antibiotics due to its ability to mask the bacterial adhesin FimH, thereby preventing its binding to urothelial cells. Three clinical trials studies revealed the efficacy of D-mannose both in reducing the symptoms and the recurrence rate of UTIs. However, the possibility that D-mannose exerts only anti-adhesive activity on FimH without altering bacterial growth and/or metabolism not have been addressed yet. Therefore, we investigated whether D-mannose might affect bacterial viability, motility, or metabolic activity of the UPEC strain CFT073. We also evaluated whether D-mannose removal after a prolonged exposure might induce mutations on FimH critical residues, thereby stably modifying FimH's ability to bind to human mannosylated proteins.

Materials and Methods. Strain CFT073 was grown at 37 C in LB or seeded onto MacConkey agar plates supplemented with different D-mannose (Utismile®, S.I.I.T) concentrations. For the evaluation of sugar metabolism and *manX* expression strain CFT073 was grown in M9 minimal medium, supplemented with D-mannose (Utismile®), D-glucose, D-fructose, and L-arabinose (Sigma). Adhesion assay was performed by infecting HTB-9 cell monolayers with strain CFT073 grown in the absence and presence of 1.5% D-mannose. The binding activity of FimH was evaluated by yeast agglutination assay. Urine glucose measurements was performed by using the Glucose Assay Kit MAK263 (Sigma).

Results. Our results show that D-mannose affected neither bacterial viability, shape, or motility nor interfered with the activity of the tested antibiotics. Moreover, in the hierarchy of *E. coli* preferred sugars, D-mannose ranked as the least preferred in supporting the bacterial metabolism and growth and its usage was prevented even after 4 h of D-glucose consumption. Since our data showed that small amounts of glucose are normally present in urine from healthy subjects, we can conclude that the dosages of D-mannose used in clinical practice are irrelevant for *E. coli* metabolism and growth. Finally, D-mannose prolonged exposure did not select FimH variants that modify bacterial adhesiveness after its removal.

Discussion and Conclusions. Overall, the low metabolic/energetic advantages for bacterial growth, the lack of selection of altered FimH adhesins after long-term D-mannose exposure, and the bladder cell tolerance emphasize the safe use of D-mannose in the treatment and prevention of UTIs caused by UPEC.

54 - Isolation of Rhodotorula spp. in enviromental samples of hospital origin

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Introduction. *Rhodotorula* species has been recognized in recent years as an opportunistic human pathogen in immunocompromised patients, leading to both systemic and localised infections such as onychomycosis and endophthalmitis. *Rhodotorula* spp. infections have been found mainly in combination with the use of invasive instrumentation such as central venous catheters (CVC), dialysis equipment, fiber optic bronchoscopes and other environmental sources. This work was conducted to assess the presence of *Rhodotorula* spp. in environmental samples of hospital origin.

Materials and methods. The environmental microbiological monitoring has been carried out during 4 consecutive years (2016/2019) in risk wards of 3 different hospital facilities located in the Province of Cagliari. Sampling involved 1,548 total samples divided as follows: 524 air samples (52 % centre chamber, 43 % vent and 5 % laminar flow hood) and 1,024 samples taken from surfaces. The sampling was performed according to the Italian ISPESL Guidelines and the samples were transported to the laboratory at refrigeration temperature (0-4° C), monitored by data logger. SDA plates were incubated at 22 ± 2° C for 72-120 h, the suspect colonies were then counted, isolated and identified (API 20C AUX Biomerieux).

Results. *Rhodotorula* spp. was isolated in 194 of 1,548 samples taken. Microbiological analysis of the air showed the positivity of 162 samples belonging to the categories "center chamber air" (63.6%) and "vent air" (36.4%) while no air sample taken inside the laminar flow hoods was found to be contaminated with *Rhodotorula* spp. *Rhodotorula* spp. was isolated more frequently ($p < 0.05$) in air samples (0.31) than in surface samples (0.03) where only 32 samples out of 1,024 were positive.

Discussion and conclusions. The results obtained showed a high frequency of *Rhodotorula* spp. isolation in the air of almost all the departments considered. *Rhodotorula* spp. is currently considered an opportunistic pathogen in immunocompromised patients, also considering its strong affinity with plastic materials (CVC, bronchoscopes, etc.), a high frequency of isolation in air can lead to an increase in risk factors with a consequent increase in the probability of contamination of surgical medical devices.

58 - Confirmed or unconfirmed cases of 2019 novel coronavirus pneumonia in Italian patients: a retrospective analysis of clinical features

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This study was aimed to compare clinical features of 165 Italian patients with laboratory confirmed or unconfirmed 2019-nCoV (also termed SARS-CoV-2) pneumonia. On March 31 2020, hospitalized patients who presented with fever and/or respiratory symptoms, exposures, and presence of lung imaging features consistent with 2019-nCoV pneumonia, were included. Before admission to a hospital ward, patients underwent RT-PCR based SARS-CoV-2 RNA detection in their nasopharyngeal swab samples. Of 165 patients studied, 119 had positive RT-PCR results and 46 were RT-PCR negative for two days or longer (i.e., when the last swab sample was obtained). The median age was 70 years (IQR, 58–78), and 123 (74.6%) of 165 patients had at least one comorbidity. The majority of patients (101/165, 61.2%) had a mild pneumonia, and the remaining patients (64/165, 38.8%) a severe/critical pneumonia. We did not find any substantial difference in symptoms, incubation periods, and radiographic/CT abnormalities as well as in many of the biological abnormalities recorded. However, at multivariable analysis, higher concentrations of hemoglobin (OR, 1.34; 95% CI, 1.11–1.65; $P = 0.003$) and lactate dehydrogenase (OR, 1.00; 95% CI, 0.99–1.01; $P = 0.05$), and lower counts of leukocytes (OR, 0.81; 95% CI, 0.72–0.90; $P < 0.001$) were independently associated with confirmed COVID-19 diagnosis. While mortality rates were similar, patients with confirmed diagnosis were more likely to receive antivirals (95% vs 19.6%, $P < 0.001$) and to develop ARDS (63% vs 37%, $P = 0.003$) than those with unconfirmed COVID-19 diagnosis. In conclusion, our findings suggest that unconfirmed 2019-nCoV pneumonia cases may be actually COVID-19 cases and that clinicians should be cautious when managing patients with presentations compatible with COVID-19.

62 - Surveillance of Legionella in two hospitals in Palermo

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Introduction Legionellae are gram negative bacteria that cause various conditions after exposure to contaminated aerosols, ranging from a serious type of pneumonia to a mild case of an influenza-like illness. Water is the major natural reservoir for Legionella, and the pathogen is found in many different natural and artificial aquatic environments such as cooling towers or water systems in buildings, including hospitals. Legionella bacteria multisystem manifestations mainly affect susceptible patients as a result of age, underlying debilitating conditions, or immunosuppression, for this reason it can be considered as responsable of nosocomial infections. *Legionella pneumophila* is the most virulent Legionella species and the most common cause of disease. However, in recent years, there were a spread of other species of Legionella, like *Legionella anisa*, in the clinic field as well as in water system. This study is focused on the evaluation of Legionella colonization of a water network of two hospitals in Palermo.

Materials and Methods From June 2018 to June 2020 an environmental surveillance was performed in two hospitals in Palermo. The water samples were collected in sterile 1-liter containers; particularly also cold and hot water were taken following the representative points of the water installation such us boiler, accomulation tank, showers and sinks of the bathrooms. The analysis was performed according to guidelines. The identification of Legionella was carried out by còagglutination test.

Results The analysis of the water network of the hospitals in Palermo revealed the presence of *L. pneumophila* sgr 1 and *L. pneumophila* sgr 2-14, often with a high load. It was also detected a little presence of *L. anisa*. In detail 47% of water samples are positive: 13% are *L. pneumophila* sgr 1, 40% are *L. pneumophila* sgr 2-14, 4% are *L.anisa* and the remaining percentage represents samples presenting coexistence of *Legionella* spp.

Discussion and Conclusion The study showed the presence of *L. pneumophila* colonization of hospital water. In hospital water systems Legionella may be resistant to disinfectants in pipework, which is a problem particularly in areas where there is low flow or stagnation of water. So periodic water sampling and active clinical surveillance in positive areas may be done to avoid nosocomial infections in hospital environment.

75 - Lack of detection of SARS-CoV-2 RNA in seminal fluid of patients affected by Covid-19.

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Introduction

Since angiotensin-converting enzyme 2 (ACE2), the main cellular receptor of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is expressed in the testis and in the male genital tract, the possibility of a testicular involvement and, thus, viral contamination of the seminal fluid have been hypothesized. Contrasting data have been reported about the presence of SARS-CoV-2 in the seminal fluid. To date, the discussion on the effects of viral infection on fertility and medicine of reproduction is still ongoing.

The aim of our study is to evaluate the possible presence of SARS-CoV-2 in semen samples of patients affected by Coronavirus Disease 2019 (COVID-19).

Materials and Methods

Semen was obtained from four SARS-Cov-2 positive patients admitted to University Hospital "Policlinico Umberto I" in Rome from April 27 through June 06. Specimens were collected manually in glass vials and processed from four to six hours later. After liquefaction at room temperature, sample from the same patient was divided to obtain total semen sample, seminal plasma and seminal cells. Viral RNA from 140 µl of seminal fluid *in toto* and from 140 µl of seminal plasma were extracted using QIAamp viral RNA kit (Qiagen), according to manufacturer's protocol; total RNA extraction from seminal cells was performed using Norgen total RNA purification kit (Norgen Biotek Corporation), according to the manufacturer's instructions. Ten µl of extracted RNA were reverse-transcribed and simultaneously amplified using a Real time RT PCR system (RealStar SARS-CoV2 RT PCR, Altona Diagnostics) targeting E and S viral genes.

Results

Four SARS-CoV-2 positive patients were recruited after informed consent and were asked to provide one ejaculated semen sample. The median age was 51.5 (IQR: 50.5-59.5) and all patients were at the acute stage of infection. No SARS-CoV-2 RNA was detected in tested semen samples.

Discussion and Conclusions

There is uncertainty regarding possible presence of SARS-CoV-2 in seminal fluid. In our study, we did not detect SARS–CoV-2 within the semen of adult males affected by COVID-19.

Despite the small sample size and selection bias due to the presence in this study of men with COVID-19 mild symptoms, this study adds information on possible male genital tract infection, virus shedding in semen, sexual transmission and safety of fertility treatments during the pandemic period. Considering the limitations, these findings must be interpreted cautiously
and further studies are required.

80 - Legionellosis risk in Palermo schools

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Introduction
Legionella is a microorganism responsible of Legionnaires' disease characterised as an acute febrile respiratory illness that often can evolve in fatal pneumonia.
The species most frequently involved in human cases is *Legionella pneumophila*, whose name literally means "lung-loving legionella".
In particular *L. pneumophila* serogroup 1 (Lp1) is the most common etiological agent causing Legionnaires' disease.
Today, *Legionella* infections are considered an emerging problem in public buildings, due to the frequent presence of the microorganism in domestic hot water, and in any situation where water stagnates a temperature of at least 25 ° C so that cases of community origin are subject to special surveillance.

Material and method
Samplings were carried out in some Palermo schools to evaluate the presence of *Legionella* spp. The presence of bacterium was sought in the samples by referring to the protocol indicated in the Italian guidelines, the operations were carried out taking all precautions, and was researched in different sampling points. The samples were collected in sterile 1-liter containers, represented by hot and cold water from the sinks of the bathrooms and offices, tanks, canteen kitchens. The cultivation investigation was carried out using BCYE agarized medium, which allows the isolation of the colonies which will appear white-gray.

Results
We analyzed five building, the analyzes showed the presence of the microorganism in three buildings. Environmental monitoring results revealed the presence of *L.pneumophila*, in particular in the first building *L.pneumophila* sgr 2-14 was found in half of the samples analyzed. In the other two buildings, fortunately, the bacterium by latex typing identified as *L.pneumophila* sgr 1, was found only in two samples.

Conclusions
The increased onset of serious epidemic episodes of Legionellosis in Italy and worldwide, together with the geographical distribution so heterogeneous of jambs belonging to the serogroup 1 of *L.pneumophila*, have necessarily highlighted the importance of the investigations. In the school buildings analyzed only in the first building the bacterial load was worrying.
However the experience gained and the feedback on the field lead to affirm that, in hot water production plants, maintenance of adequate temperatures and the meticulous cleaning of the water storage tanks, of the boilers, of the pipes, can allow the containment of the levels of contamination.

84 - Viral co-infections in acute pediatric gastroenteritis over11 years in Palermo

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Introduction:Acute gastroenteritis (AGE) is one of the most common illnesses inhumans worldwide, being responsible for half a million deaths amongchildren aged<5 years, mostly in developing countries. Four viruses are recognized as etiologic agents of AGE: group A rotavirus (RVA), norovirus (NoV), adenovirus 40/41 (AdV) and astrovirus (AstV).The introduction of multiplex PCR diagnostic tools for the diagnosis of enteric infections has improved the laboratory workflow and has also unveiled the phenomenon of co-infections. This study was carried out to assess the impact of RVA, NoV, AdV and AstV co-infections in children hospitalized for AGE and to definethe genetic diversity of such viruses.

Materials and methods: A total of 4161 stool samples were collected from diarrheic children hospitalized for AGE at the "G. Di Cristina" Children Hospitalof Palermo, from January 2008 to December 2018. All sampleswere initially screened for the presence of RVA, NoV, AdV40/41 and AstVby either molecular or immunochromatographic assays and positive samples were subsequently confirmed by Real-Time PCR and sequence analyses.

Results: At least one of the four viruses was detected in 48.6 % of specimens and 8.3 % was co-infected with either two or three different viruses. RVA was the most prevalent mono-infection in symptomatic children (24.7 %) followed by NoV GII (18.9 %), AdV (5.3 %) and AstV (2.9 %).
Most of the co-infections involved RVA, mainly occurring with NoV (67.7 % NoV GII and 3% NoV GI), followed by the RVA/AstVcombination (9.6 %). The highest rate of association to mixed infectionswas observed for AstV(26 %), followed by NoV GII (16.3), RVA (13.9 %) and AdV (7.7 %).

Discussion and Conclusions: The recent development of multiplex biomolecular tests is useful to improve diagnostic performance as it allows to detect underestimated viruses and define the epidemiological trend of gastrointestinal disease. However,in the case of co-infections it could be difficult to attribute the exact role to each pathogenor speculate synergistic enhancement of the severity of the disease.Future studies combining clinical and epidemiological information will be essential for a more accurate evaluationof disease etiology in symptomatic patients.

88 - Research of SARS-CoV-2 in semen in Italian males recovering from mild COVID-19

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Introduction: Pandemic Coronavirus Disease 2019 (COVID-19), due to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral infection, have involved millions people globally becoming a serious worldwide public health emergency. Droplets and fomites are generally considered the most important transmission route of COVID-19, but little is known about the possibility of sexual transmission. Up to present few studies looking for the presence of SARS-CoV-2 in human semen are available but come to discordant conclusions. The aim of this study was to determine if SARS-CoV-2 RNA is detectable in human semen of patients with positive nasopharyngeal swab test and showing mild symptoms of COVID-19.

Materials and methods: Semen samples were collected from patients with positive nasopharyngeal swab PCR tests for SARS-CoV-2 (performed according to WHO guidelines) and isolated in non-hospital settings (e.g. houses or hotels). Information about comorbidities, therapy and urological conditions were collected. Ejaculated semen specimens of 5 healthcare workers testing negative for blood serum anti-COVID-19 IgM and IgG were used as control group. Viral nucleic acids were extracted from semen samples and the detection of SARS-CoV-2 was performed using real-time RT-PCR protocols targeting three different genes: viral RNA polymerase (R), envelope protein (E) and nucleocapsid protein (N). All PCR tests were performed in duplicate and a cycle threshold value less than 40 cycles was defined as a positive test.

Results: Out of the 29 patients initially contacted and eligible, only 9 gave their written informed consent to participate and were enrolled to this study. The 9 patients enrolled for semen testing had all been positive at SARS-CoV-2 nasopharyngeal swabs at least once, but seven of them had been tested positive more than once. Semen sampling from of all 9 patients was performed within 13 days from their last positive nasopharyngeal swab RT-PCR test result (range, 4-13 days; median, 7 days), and when they mostly had no symptoms. Results of real-time RT-PCR in semen showed no evidence of SARS-CoV-2 RNA in the 9 patients, being their samples negative for the detection of all the three molecular targets used in duplicate repetitions. As expected, also the 5 healthcare workers used as control group were tested negative for SARS-CoV-2 in semen.

Discussion and conclusions: Although it seems very unlikely that SARS-CoV-2 can be sexually transmitted by men who are recovering from mild symptoms of COVID-19, the presence of SARS-CoV-2 in seminal fluid during severe acute COVID-19 cannot be excluded. Further large-scale studies are needed to confirm or exclude the possibility of sexual transmission of SARS-CoV-2, which could have important clinical and public health implications.

90 - Clostridium cadaveris Bacteremia

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Introduction: *Clostridium cadaverisi*s a strict anaerobic Gram-positive bacillus commonly found in the gastrointestinal tract of humans and other mammals. This non-toxin producing clostridia species was identified as the microbial species responsible for gaseous production during human decomposition. Although present in the intestinal flora of humans, *C. cadaveris* is rarely responsible for infectious pathology in living patients.

Materials and Methods: Blood cultures were incubated in the BACTEC System. The FilmArray Blood Culture Identification (BCID) Panel simultaneously tests the positive blood culture sample to provide results for 24 different organisms (but no anaerobic bacteria) and organism groups that cause bloodstream infections. Anaerobic subcultures were carried out on blood agar and Schaedler's medium using standard procedures. Identification of C. cadaveris can be established using standard laboratory techniques, based on the specific characteristics of the organism. The ANC Card Vitek 2 Compact (BioMerieux) gave excellent identification of C. cadaveris (ID 99.9%). Susceptibility testing was performed by Kirby Bauer Test.

Results: A 56-year-old, male with ulcerative e putrefaction wound post-surgery presented to the emergency department on 25.02.2020 with complaints of fewer, asthenia, tachycardia and hypotension. Dagli esami ematochimici: ↑ Leucociti 20.50 mila/µL (v.n. 4.0-10.0), soprattutto Neutrofili 19.08 mila/µL (2.0-8.0) e Proteina C Reattiva 31.68 mg/dL (v.n. 0.0-0.5). On 26.02 esame colturale ferita (effettuato in altro laboratorio) negativo. On 27.02 Emocoltura positiva processata con FilmArray (negativa) e semina tradizionale. On 29/02 Subcoltura Emocoltura positiva per C. cadaveris. Paziente sottoposto a debridment chirurgico del tessuto necrotico e a terapia antibiotica. Paziente muore per sepsi da C. cadaveris il 03.03.

Discussion and Conclusions: C. cadaveris is rarely found in clinical specimens. This case demonstrates that C. cadaveris can be a pathogen in a patient without any underlying malignancy or other cause of immunosuppression who has severely infected wounds. We emphasize the importance of anaerobic cultures for septic patient because the incidence of anaerobic wounds infections is uncommon to rare. Success rates vary as do the antibiotic susceptibilities of responsible microbes. Reasons for the difficulty at eradication of Clostridium cadaveris remain unclear but may be due to antibiotic delivery issues, bacterial killing potential of antibiotics, and biofilm characteristics.

93 - Salmonella typhimurium Empyema

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Introduction: Salmonella spp. Are Gram-negative, non-spore-forming, facultative anaerobic bacilli of the family Enterobacteriaceae. Non-typhi Salmonella enterica serovars most commonly present with gastroenteritis. Non-typhi S. enterica serovars rarely cause pleuropulmonary disease, in particular pleural empyema. This case report presents a case of unilateral empyema caused by Salmonella enterica serovar typhimurium

Materials and Methods: The pleural fluid is inserted into enrichment vials and placed in the Bactec. When positive, subcultures are performed on traditional agar. Lactose-free fermenting colonies on MacConkey Agar are identified at Vitek 2 Compact and subjected to antibiotic sensitivity testing. Simultaneously agglutination tests are performed with polyvalent and monovalent antisera for Salmonella to identify the serovar typhimurium (positive test for Poly A-I, O4,O12).

Results: A 55-year-old male presented to the emergency department on 11.07.2020 with a 2-day history of increasing right-sided pleuritic chest pain associated with increased shortness of breath and increased non-productive cough. There was no fewer or inreased sputum production. He report profuse diarrhea and flank pain on admission. Chest radiograph on presentation showing evidence of right-sided pleural effusion. From blood chemistry tests: Normal White Blood Cell count, Bilirubin 7.36 mg/dL (v.n. 0.3-1.2) and increased LDH 510 U/L (v.n. 125-220). A Thoracocentesis was performed on 12.07. On 13.07 subsequent cultures showing Gram-negative bacilli. The day after grew S. enterica serovar typhimurium. The patient started targeted antibiotic therapy. He is currently hospitalized in the medical ward.

Discussion and Conclusions: Salmonella empyema is an uncommon presentation, with only few case reports published in the last century. It can be a difficult diagnosis, with a lack of raised leucocytes, or a lack of positive blood cultures to assist with diagnosis. Therefore, although rare, Salmonella as a cause of pleural effusion should remain a differential diagnosis. The multidisciplinary approach remains necessary for a timely diagnosis.

99 - Inhibition of SARS-CoV-2 infection by a highly potent lipid-conjugated peptide derived from its Spike Protein

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Introduction The novel coronavirus SARS-CoV-2 that emerged in late 2019 from Wuhan, China, has rapidly spread throughout the world, that has led to tens of millions of infections and hundreds of thousands of deaths worldwide. The development of therapeutics to treat infection or as prophylactics to halt viral transmission and spread is urgently needed. Here, we describe the development of a lipopeptide, derived from the C-terminal heptad repeat (HRC) domain of SARS-CoV-2 S, that potently inhibits infection by SARS-CoV-2.

Materials and Methods Peptide was tested *in vitro* for inhibitory fusion activity by fusion assay based on alpha complementation of β-galactosidase (β-Gal) and for inhibitory activity against SARS-CoV-2 by pseudotype viral assay.

Results The lipopeptide inhibits cell-cell fusion mediated by SARS-CoV-2 S and blocks infection by SARS-CoV-2 S mediated infection.

Discussion and Conclusions These data provide a framework for the development of peptide therapeutics for the treatment of or prophylaxis against SARS-CoV-2.

100 - Vasculitis and cardiovascular damage secondary to Sars-CoV-2 infection: a case report

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Introduction.
Sars-CoV-2, is a novel RNA virus that causes acute pneumonia that can lead to severe respiratory distress syndrome, which is sometimes fatal. Some individuals become infected but do not develop any symptoms. Most currently confirmed cases - especially children and young adults - appear to have mild, flu-like, and slow-onset disease.
We report a case of vasculitis and cardiovascular damage secondary to Sars-CoV-2 infection with recurrently positive genome SARS-CoV-2 from an oropharyngeal swab test.

Case presentation
A clinical case of a 82-year-old woman with an history of chronic renal impairment and who has undergone dialysis treatment for years is presented. She came to our Emergency Department on April for a revision of arteriovenous fistula. She underwent an oropharyngeal swab test for SARS-CoV-2 genome research resulting positive. Upon access to the emergency room she did not present other symptoms, the next two oropharyngeal swab tests performed during hospitalization were negative and the patient was dismissed. A month later, the patient came to the emergency room for petechial skin lesions and a clinical diagnosis of cutaneous vasculitis was made. The oropharyngeal swab test tested positive for Sars-CoV-2 genome research.
She was hospitalized and subjected to skin, vascular biopsy and blood and urine tests. Laboratory testing showed a normal white blood cell count, thrombocytopaenia was not observed, but the inflammation indexes were altered. PCR for SARS-CoV-2 in vascular biopsy was negative. Serological testing for SARS-COV-2 was positive for IgA and IgG antibodies. After stabilization of her clinical condition, the patient was discharged following two consecutive tests of oropharyngeal swab negative for Sars-CoV-2. On June, the patient was hospitalized for acute myocardial infarction and positivity for Sars-Cov-2 at the oropharyngeal swab test was detected. After the interventional cardiological procedure, however, vascular instability remains that will lead to death.

Discussion and Conclusions
Vasculitis is the result of inflammation of the walls of blood vessels resulting from the deposition of immune complexes that activate the complement cascade. The elevation of IL-6 has been associated with the large cytokine storm that causes a serious infection with COVID-19. In the three acute episodes of vascular damage (inflammation of the arteriovenous fistula, vasculitis and myocardial infarction) PCR is elevated in conjunction with the positivity for SARS-COV-2.

We postulated that all episodes of vascular damage in our patient may have been caused by an immune response against viral antigen deposition or as a consequence of elevating IL-6 during infection, which may have produced endothelial damage.

102 - COVID-19 disease severity and its association with SARS-CoV-2 "viral load" at diagnosis: single-centre experience.

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Introduction
In January 2020, the 2019 novel coronavirus (SARS-CoV-2) was confirmed as the cause of pneumonia cases of unknown origin emerged in December in Wuhan. The disease associated to SARS-CoV-2 infection shows a wide spectrum of clinical severity, but the role of viral dynamics and the mechanisms involved in this variability are still poorly understood.
Therefore, the objective of this study was to investigate the association of COVID-19 severity in SARS-CoV-2 infected patients with clinical and virologic parameters, and particularly in relation to viral load (VL) in nasopharyngeal swabs (NPS) at hospital admission.

Materials and Methods
The study included 176 patients diagnosed with SARS-CoV-2 infection. Virus detection was performed on NPS by RNA extraction (Versant SP 1.0 Reagents kit, Siemens) followed by a real time RT-PCR assay detecting the E and S genes of SARS-CoV-2 (RealStar SARS-CoV-2 RT-PCR, Altona).
Demographic and clinical data of patients were acquired from clinical records. According to COVID-19 severity, patients were classified in 3 groups: asymptomatic/mild (n=44), moderate (n=55) or severe (n=77). Among severely ill patients, 79.2% (61/77) died from the infection.
Mann–Whitney U and Chi-squared tests were used to compare VL, demographic and clinical parameters among groups. Statistical tests were conducted two-sided at a significance level of 0.05.

Results
Most of patients included in the study were males (119/176, 67.6%). Median age of patients was 67 years (IQR: 55-76). Both gender and age distributions were associated to severity: the percentage of male patients increased from 54% to 78% between asymptomatic and severe groups, and median age was significantly higher among severe patients (73 years, IQR: 66-82) compared with those with moderate (64 years, IQR:58-75; $p<0.05$) and asymptomatic disease (56 years, IQR: 45-68; $p<0.0001$). Cycle threshold (Ct) values of both E and S genes in NPS of severe group were significantly lower than those detected in asymptomatic/mild and moderate group ($p<0.05$), suggesting a relationship between VL at diagnosis and the disease outcome. The duration of hospitalization and the time from symptoms onset to hospital discharge significantly increased following the degree of severity. Compared to patients with asymptomatic/mild disease, clinical parameters of moderate and severely ill patients varied significantly, with lower P/F ratio, higher white blood cells and neutrophils counts, higher values of lactate dehydrogenase and D-dimer.

Discussion and Conclusions
Our data indicate that patients with severe COVID-19 have a higher VL compared to patients with milder disease, suggesting that VL at baseline could support clinical evaluation of patients as a marker of disease progression and prognosis.

108 - Enhanced binding of SARS-CoV-2 Envelope protein to tight junction-associated PALS1 could play a key role in COVID-19 pathogenesis

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The Envelope (E) protein of SARS-CoV-2 is the most enigmatic protein among the four structural ones on the viral genome. Most of the current knowledge on the E protein is based on the direct comparison to the SARS E protein, initially mistakenly undervalued and subsequently proved to be a key factor in the ER-Golgi localization and in tight junction disruption. When compared to the known SARS E protein, we observed a different amino acidic sequence in the C-terminal of the E protein of SARS-CoV-2 which might have a key role in the current COVID-19 pathogenesis. By *in silico* modelling analyses of protein conformation and docking, we suggest that SARS-CoV-2 E protein may interfere with the tight junctions stability and formation leading to an enhanced epithelial barrier disruption, amplifying the inflammatory processes and promoting tissue remodelling. These findings raise a warning on the underestimated role of the E protein in the pathogenic mechanism and could open the route to detailed experimental investigations.

109 - The transcriptome of Trichomonas vaginalis in symbiosis with Mycoplasma species: a deep analysis to characterize these intriguing relationships.

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Introduction: *Trichomonas vaginalis*, a mucosal parasite of urogenital tract, has been shown to be closely associated with two *Mycoplasma* species: *Mycoplasma hominis* and *Candidatus Mycoplasma girerdii*, recently characterized through metagenomic analysis. Several articles have reported the influence of *M.hominis* on pathobiology of *T.vaginalis*, suggesting an endosymbiotic nature of this relationship. Although the relative risk for co-occurrence of '*Ca.M.girerdii*' with *T.vaginalis* is higher than *M.hominis* suggesting a stronger association, there is very few information on the nature and impact of this new symbiosis on the pathobiology of the protist.
In the current work, we provide to investigate the transcriptional mechanisms underlying the interaction between *T.vaginalis* associated both *Mycoplasma* species, by RNA-Seq analysis of protozoan isolates harbouring intracellular *M.hominis* and '*Ca*.M.girerdii'.

Materials and Methods: An *in vitro* model system of single and double infection of mycoplasma-free *T.vaginalis* recipient was developed starting from axenic *T.vaginalis* cell culture. mRNA extraction was performed from protozoa and bacteria, and libraries were analyzed by Illumina sequencing.

Results: *T.vaginalis* reads were the most abundant in all libraries, with endosymbiont '*Ca.M.girerdii'* reads more abundant in the *T.vaginalis*-'*Ca*.M.girerdii' association compared with *M.hominis* reads found in *T.vaginalis* parasitized by *M.hominis*. A largely overlapping set of genes appears to be upregulated in response to *Mycoplasma* species in trichomonads. Moreover, a large set of genes was uniquely regulated in response to single mycoplasma infection. The presence of distinct *Trichomonas vaginalis* viruses was also identified from *T.vaginalis* strains.

Discussion and Conclusions: RNA-Seq analysis has shown that the presence of single or double mycoplasma infection in *T.vaginalis* cells can modulate the protozoan gene expression. Interestingly, '*Ca.M.girerdii'* seems to have a much larger impact on the transcriptional response of protozoon than *M.hominis*. Moreover, the presence of both *Mycoplasma* appeared to have an influence on the relative abundance of viral transcripts. The comparison among the transcriptomes of *T.vaginalis* mycoplasma-free and *T. vaginalis* associated with *Mycoplasma* species has further highlighted the important role of symbionts in trichomonad biology.

128 - Long-term SARS-CoV-2 infection associated with viral dissemination in different bodily fluids including bile in two patients with acute cholecystitis

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Introduction: It is known that the main site of infection and replication of SARS-CoV-2 is the respiratory tract. Despite this, several studies have detected the virus in extra-pulmonary body districts, in particular in the cells lining the gastrointestinal tract, suggesting the role of digestive system as a route of infection. In this light, we investigated the detectability and kinetics of SARS-CoV-2 in different bodily fluids, including the bile, of two SARS-CoV-2 positive patients with acute cholecystitis, treated conservatively with a cholecystostomy.

Materials and Methods: For each patient, nasopharyngeal and rectal swabs, urine, bile and plasma samples were collected at different time-points during their hospitalization. SARS-CoV-2 genome was detected and quantified in the all biological samples by two droplet digital PCR (ddPCR) assays, targeting the genes encoding viral RdRp and nucleocapsid.

Results: We reported two SARS-CoV-2 infected patients with acute cholecystitis, treated conservatively with a cholecystectomy. For both patients, ddPCR assays revealed persistent and prolonged detection of SARS-CoV-2 load in nasopharyngeal swabs despite triple-negative or single-positive results by Real-Time PCR. Interestingly, SARS-CoV-2 viral load showed different kinetics according to the bodily fluids analyzed. In particular, by focusing on Patient 1, viral load decreased more rapidly in bile and rectal swabs while it slowly declined in nasopharyngeal swabs and plasma resulting undetectable 97 days after the onset of symptoms. Conversely, in patient 2, SARS-CoV-2 load was detected, even if at low copies, in all bodily fluids (including bile) up to 76 days after the onset of symptoms when the patient was discharged. The only exception concerned the urine, in fact, the viral load in this kind sample was detected at low levels only at first time point.

Discussion and Conclusions: These findings support the higher sensitivity of ddPCR for clinical detection and quantification of SARS-CoV-2 respect to Real-Time PCR. Moreover, this study shows that SARS-CoV-2 genome can persist for prolonged time not only in respiratory samples but also in several biological samples despite negativity to Real-Time PCR, supporting the SARS-CoV-2 ability to give origin to persistent and disseminated infection and therefore to contribute to extra-pulmonary clinical manifestati

140 - The cholesterol metabolite 27-hydroxycholesterol inhibits SARS-CoV-2 and is markedly decreased in COVID-19 patients.

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Introduction There is an urgent need to identify antivirals against the human coronavirus (HCoV) SARS-CoV-2 in the current COVID-19 pandemic and to contain future similar emergencies early on. Specific side-chain cholesterol oxidation products of the oxysterols family have been shown to inhibit a large variety of both enveloped and non-enveloped human viral pathogens. In this study we focused on investigating the anti-CoV potential of the oxysterol 27-hydroxycholesterol (27OHC), a cholesterol oxidation product of enzymatic origin, constitutively present in several human biologic fluids, and capable of inhibiting or even preventing the infection of both enveloped and non-enveloped viral pathogens.

Materials and Methods We tested the in vitro antiviral activity of 27OHC against a clinical isolate of SARS-CoV-2 and a second member of beta-CoV subfamily and common cold etiological agent, OC43, by plaque reduction assay. Importantly, we integrated these in vitro data with relevant clinical evidences, by assessing the oxysterols hematic profile in 67 consecutive Covid-19 adult patients. Only SARS-CoV-2 cases confirmed through real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays of nasal and pharyngeal swabs were included in the analysis.

Results The results of in vitro antiviral assays demonstrate the in vitro inhibitory activity of the redox active oxysterol 27OHC against SARS-CoV-2 and against HCoV-OC43 without significant cytotoxicity. Interestingly, physiological serum levels of 27OHC in SARS-CoV-2 positive subjects were significantly decreased compared to the matched control group, reaching a marked 50% reduction in severe COVID-19 cases. Moreover, no correlation at all was observed between 24-hydroxycholesterol and 25-hydroxycholesterol serum levels and the severity of the disease. Opposite to that of 27OHC was the behavior of two recognized markers of redox imbalance, i.e. 7-ketocholesterol and 7β-hydroxycholesterol, whose serum levels were significantly increased especially in severe COVID-19 patients.

Discussion The exogenous administration of 27OHC may represent in the near future a valid antiviral strategy in the worsening of diseases caused by present and emerging CoVs.