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POSTERS

Poster 1

The effect of 2,3-diphosphoglycerate (2,3-DPG) on malaria parasite development – is there a potential for a host-directed therapy tool?

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Background: The fight against malaria, one of the top 10 causes of death worldwide, remains a challenge due to the lack of an efficient vaccine and emergence of resistant parasites to antimalarial drugs. Yet, parasite dependence on red blood cells (RBC) may provide targets for new approaches. Malaria infection has exerted a strong selective pressure on the human genome, resulting in human variants that may protect against the disease, such as the RBC pyruvate kinase deficiency (PKD). Intraerythrocytic increase of 2,3-DPG metabolite, and decrease of ATP production are consequences of PKD and could be involved in this protective effect. On the other hand, Plasmodium falciparum-infected normal RBC (iRBC) show a decrease of 2,3-DPG and increase in ATP production due to the activity of parasite's PK enzyme. We hypothesised that RBC glycolysis could be a target to fight malaria and the first approach to test it was to assess the effect of exogenous administration of 2,3-DPG to iRBC in vitro.

Methods: Synchronized cultures of ring-stage P. falciparum 3D7 (iRBC) were used to perform parasite susceptibility assays through determination of IC50 values and invasion and maturations ratios during 2-3 parasite life cycles (96-144h). Simultaneously, toxicity for human cells was assessed through MTT assays on HepG2 cells incubated with 2,3-DPG for 48 hours.

Results: IC50 values increased on the first cycle of 48h in 2,3-DPG-treated iRBC, while the opposite was observed during the second cycle (from 48 to 72h). Lower maturation and invasion ratios were observed in 2,3-DPG treated iRBC. IC50 values obtained on MTT assays indicate a possible safe use of 2,3-DPG in higher doses than the physiological one.

Conclusions: These preliminary results seem to indicate that 2,3-DPG added to P. falciparum in vitro cultures interferes with the parasite development, as showed by a lower maturation and invasion ratios. Different values of IC50 through the parasite life cycle suggest that the effect of 2,3-DPG varies according to the parasite stage, which may be related with the metabolic rate of different stages.

Poster 2

Creating value in health sciences at NOVA – implementation of biobanks in Infectious and Chronic Diseases

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Research collaboration in chronic and infectious diseases promotes research excellence and global health improvement. Biobanks are considered key structures in supporting biomedical research assuring high-quality biospecimens collected from patients (or from healthy donors) along with respective clinical data and participant consent and made available for research purposes.

Two schools of NOVA University have implemented local biobanks in Infectious Diseases – Biotropical Resources (BIOTROP) at GHTM/IHMT and in Chronic Diseases – Comprehensive Health Research Centre (CHRC-Biobank), at CEDOC/NMS.

BIOTROP was created in 2016 to improve research in tropical medicine and infectious diseases. BIOTROP's collections are heterogeneous, ranging from pathogens of human and companion animals' origin to their vectors and reservoirs. BIOTROP is prepared to receive, preserve, and distribute the biological products, strictly following the legal and ethical issues, Nagoya protocol and the international best practice guidelines for these infrastructures. Data management and confidentiality good practices were recognized by the Data Privacy Impact Assessment evaluation performed by NOVA Data Protection Officer (2019).

The CHRC-Biobank´s mission is supporting biomedical, translational, and clinical research activities carried out at CEDOC and iNOVA4Health, related to chronic diseases such as musculoskeletal, cardio, respiratory disorders, and mental health. One of its main assets are the collections from national cohorts, namely EpiReumaPt (part of EpiDoC, a population-based cohort representative of the Portuguese population). CHRC-Biobank will also offer services as: Standard operating procedures (SOPs), quality control of biospecimens and procedures, data protection policies and adherence to the national and international guidelines and laws.

BIOTROP and CHRC-Biobank are part of Portuguese Roadmap of Research Infrastructures and National Biobanks Infrastructure. BIOTROP is also member of the Portuguese microBiological Resources Center Network (Pt-mBRCN). Together, they constitute a major asset at NOVA to strengthen research quality and encourage national and international collaborations.

Poster 3

Protective effect of red blood cell pyruvate kinase deficiency against malaria – is there a role for the host cell membrane?

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Background: Malaria is caused by Plasmodium parasites and spread by mosquitoes. It is a major public health problem as insecticide-resistant mosquitos and drug-resistant parasites continuously emerge and rapidly spread worldwide. It is reckoned as one of the strongest driving forces on the human genome, selecting for genetic variants that may provide some protection against disease severity. If we understand the underlying mechanisms, we may try to develop new approaches to fight this disease. Individuals with red blood cell (RBC) pyruvate kinase deficiency (PKD) show a decrease in intraerythrocytic ATP and an increase of the metabolite 2,3-diphosphoglycerate (2,3-DPG). Our previous studies showed that exogenous administration of 2,3-DPG to parasite cultures seriously affect parasite development, with a 2,3-DPG dose-dependent lower parasitaemia.

Objectives: We hypothesised that parasite invasion or egress of RBC may be the main affected parameters and we aimed to evaluate the effect of 2,3-DPG on RBC membranes.

Methods: To characterize changes on RBC viscoelastic, biomechanical and membrane potential properties and to analyse if these changes could impair P. falciparum invasion, growth or egress, non-parasitized (niRBC) and parasitized RBC (iRBC) from synchronized P. falciparum 3D7 cultures, cultivated in the presence and absence of 2,3-DPG, 8mM for 30h, were used to perform atomic force microscopy (AFM) and zeta potential (ZP) measurements. Blood smears were made throughout the entire cycle to monitor parasitaemia.

Results: The addition of 2,3-DPG to the culture medium does not seem to affect RBC stiffness or deformability, but it seems to increase the membrane ZP of niRBC. Blood smears show a delay in parasite development in iRBCs and clearly show that, after the cycle is completed, parasitaemia decreases drastically.

Conclusions: These preliminary results hint that 2,3-DPG changes the zeta potential of niRBC. Considering that the parasite is able to fully develop, even if at a slower pace, the decrease in parasitaemia most likely results from an impaired egress from the infected host cell and/or a failure to infect a new RBC whose surface charge was altered.

Poster 4

Real-time efflux activity in clinical isolates of Escherichia coli can be evaluated using Hoechst 33258, a bis-benzimidazole derivative

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The global spread of bacterial multidrug resistance (MDR) is a serious threat to public health leading to an increase in mortality and morbidity as the result of an ineffective therapy. Escherichia coli drug resistance has become a worldwide issue often due to the presence of MDR efflux pumps (EPs). Here we aim to evaluate the applicability of Hoechst 33258, a fluorescent molecule less toxic than ethidium bromide, for the characterization of efflux activity in Escherichia coli.

We tested a panel of 27 E. coli clinical isolates, from different Portuguese health care units, 3 control strains that differ on its efflux activity (AG100, AG100A and AG100tet) and the reference strain ATCC25922. Antibiotic susceptibility testing was performed using Kirby-Bauer disc diffusion method. Determination of minimum inhibitory concentrations (MICs) of antibiotic, biocides and dyes was evaluated in presence and absence of efflux inhibitors (EIs). Quantification of efflux activity was measured through accumulation and efflux assays of Hoechst 33258 using for this a 96-well plate semi-automated fluorometric method.

Among the isolates studied, 12/27 were MDR. All isolates show resistance to β-lactams but only one strain show resistance to extended-spectrum cephalosporins. MDR isolates presented resistance to quinolones, chloramphenicol, tetracyclines and/or aminoglycosides. Resistance to carbapenems and tigecycline was not detected. Among the 4 EIs tested (PAβN, CCCP, NMP and CPZ), PAβN was the one the most efficient in reducing the MICs of antimicrobials. The EIs that promote the most significant inhibition of efflux of Hoechst 33258 in real-time were CPZ and CCCP.

This work demonstrate that is possible to differentiate E. coli strains with different levels of efflux using Hoechst 33258. Being efflux, an important contributor to the development of MDR phenotypes in E. coli, this work highlights the relevance of combined therapy to tackle these infections. In this sense, the use of new fluorescent molecules as Hoechst 33258 will allow increasing our knowledge about the activity of efflux systems as well as to identify new substrates and inhibitors being a value tool for the development and implementation of new therapeutic strategies.

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Poster 5

Optimization of a cost-effective method for the simultaneous detection of β-lactamases and efflux activity in Escherichia coli

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The acquisition of β-lactamases is one of the main mechanisms described for emergence of antimicrobial resistance in Enterobacteriaceae, and recent work performed by our group demonstrates that efflux contributes this resistance phenotype. Here, we aim to establish a cost-effective method for the fast screening of β-lactamases, extended spectrum β-lactamases, metallo-β-lactamases (MBLs), and carbapenemases and the simultaneous presence of efflux activity in Escherichia coli.

We studied 27 clinical isolates of E. coli from Lisbon hospital units. Molecular typing using ERIC-PCR, drug susceptibility testing by disc diffusion and the determination of minimum inhibitory concentrations (MICs), for antibiotics and efflux inhibitors, were performed. MICs of β-lactams in presence and absence of β-lactamase-specific inhibitors, and MICs in presence and absence of efflux inhibitors in combination with β-lactamase-specific inhibitors, using a 96-well broth microdilution MTT-based method, were also determined. The results were validated using phenotypic standard methods for β-lactamases detection and screening of genes encoding chromosomal and plasmid-encoded β-lactamases by PCR.

β-lactamase production was detected in all isolates and included the presence of AmpC TEM with OXA-1, SHV, or CTX-M or AmpC overexpression and/or plasmid-mediated AmpC production. Carbapenemases and MBLs were not detected. We were able to reduce the MICs of β-lactams in presence the efflux inhibitors in combination with β-lactamase-specific inhibitors, indicating the existence of active efflux. Comparing the commercial vs non-commercial assays, we observed that the non-commercial assays performed better than the commercial assays.

In this work, we optimize a cost-effective method for the simultaneous and fast screening of β-lactamases and efflux activity, which may be a promising alternative to the diagnostic techniques currently available. This easy-to-perform approach has potential for being implemented in routine laboratories, contributing to the rapid detection of β-lactam resistance and efflux activity. This work will be extended to further Enterobacteriaceae clinical isolates for validation and determination of performance parameters.

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Poster 6

Microfluidics production of enzyme-loaded liposomes towards the treatment of inflammatory diseases

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Framework: Biopharmaceutics (BF) are one of the fastest growing areas on the pharmaceutical industry. Despite their advantages over the conventional drugs, BF have short biological half-lifes, which can be increased using a drug delivery system. Liposomes are constituted by a lipid bilayer, enclosing an inner aqueous core. These physicochemical features enable liposomes to carry both hydrophobic and hydrophilic active compounds, enhancing the blood residence time and the targeted delivery of the BF. However, the common bulk methods to produce BF-loaded liposomes result in loss of encapsulation efficiency (E.E.), resulting in an expensive process. Objectives: To design and develop an efficient method to produce BF-loaded liposomes with higher encapsulation efficiency and therapeutic effect in an inflammatory environment. Method: We proposed the encapsulation of an enzyme with anti-inflammatory properties in liposomes, using a glass-capillary microfluidic technique. Results: The enzyme was successfully encapsulated into liposomes. Enzyme-loaded liposomes with a mean size of 135±41 nm, a polydispersity index of 0.13±0.01, an E.E. of 59±6 % and an enzyme activity of 82±3% were obtained. In vivo experiments showed that the enzyme-loaded liposomes administered by the intravenous route enabled an edema inhibition of 65% ± 8%. Conclusion: This work highlights the potential of microfluidics for the production of enzyme-loaded liposomes, with high encapsulation efficiency and therapeutic effect in an inflammatory environment.

Poster 7

Biochemical characterization of Neisseria gonorrhoeae cytochrome c2 and nitrite reductase involved in peroxide detoxification and denitrification

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Neisseria gonorrhoeae is an obligate human pathogen responsible for the sexually transmitted disease gonorrhea that affects millions of people worldwide. Its infection site presents a primarily anaerobic microbiota that acts as a defensive reinforcement against infectious agents through the production of reactive oxygen (ROS) and nitrogen species, namely H_2O_2 and NO[1,2]. Thus, the ability to grow under anaerobic conditions and detoxify ROS is essential for N. gonorrhoeae survival in the host.

A periplasmic truncated denitrification pathway comprising a nitrite reductase (AniA) and a nitric oxide reductase has been proposed as the anaerobic respiration pathway, as well as a NO_2^- and NO detoxifying system[3]. Moreover, a periplasmatic bacterial peroxidase (CCP) that reduces H_2O_2 to water has been reported to act as a resistance mechanism against peroxidative stress[3].

The periplasmatic cytochrome c2 (Cytc2), a putative electron donor for AniA and CCP, and AniA were heretologously produced in Escherichia coli. Cytc2 coordination sphere and folding were assessed by 1H-NMR, identifying a methionine residue as the distal axial ligand of the c-type heme. Circular dichroism allowed to evaluate its secondary structure and thermostability, estimating a denaturation enthalpy (ΔH) and melting temperature (TM) of 157 kJ/mol and 61 °C, respectively. The reduction potential was determined by cyclic voltammetry to be +159 mV (vs SHE) pH 7.0.

AniA was isolated with two copper ions per monomer and its UV-visible spectra indicates that it belongs to the blueish-green class of copper nitrite reductases. The catalytic activity of AniA was assayed using the artificial electron donor methylviologen. Preliminary kinetic assays with Cytc2 and a lipid-modified azurin (Laz) suggests the ability of Cytc2 and the inability of Laz to donate electrons to AniA.

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Poster 8

Assessment of the safety and therapeutic benefits of convalescent plasma and hyperimmune plasma in COVID-19 treatment: a systematic review

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The newly emerging coronavirus disease (COVID-19), caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), prompted a global health crisis. To date, there are no specific treatments to prevent its dissemination. Convalescent plasma (CP) is as a commonly employed therapeutic approach for infections with rapid evolution and deemed as safe. CP has been purposed to treat and contain SARS-CoV-2 infection. However, concerns about its safety and efficacy have kept COVID-19 CP as an investigational product, not yet approved by FDA nor EMA. While multiple studies are now reported, consistent and randomized controlled trials (RCT) to assess its potential therapeutic effect are limited. Yet, it is relevant to objectively analyse the data, and quality of those RCT to understand not only CP therapeutical potential, but also to select the patients with higher chances to benefit, expected outcomes, among other clinical parameters. To assess this, we performed a systematic review to analyse the published clinical trials using CP and hyperimmune plasma (fractioned CP product with higher antibody concentration) for COVID-19 treatment. Multiple aspects of the trials such as mortality rates, safety of the intervention and improvement of the clinical outcomes were assessed.

To perform this systematic review, we selected keywords to search and retrieve information from multiple Databases (e.g Pubmed, B-on, SCOPUS). After organizing, rating and/or excluding the studies collected, it remained 7 clinical studies (2 RCT and 5 controlled non-randomized clinical trials (CNRCT)). We then extracted the relevant information (from patient baseline characteristics to intervention methods) to a previously developed datasheet to analyse results both quantitative and qualitatively. The preliminary data comparing CP intervention with standard treatments indicate that CP does not favour a decrease in mortality, but it has a positive impact in reducing the viral load from the organism. The qualitative analysis to the existence of bias (systematic error or deviation from the truth, which involves assessing multiple conditions) indicates that the RCT studies present an overall high risk of bias, and the CNRCT studies present an overall high to critical risk of bias. In conclusion, CP is a potentially efficacious treatment for COVID-19 but further studies are necessary to draw tangible conclusions about mortality.

Poster 9

Targeting Mycobacterium tuberculosis membrane energetics with lipophilic efflux inhibitors as adjuvants of tuberculosis treatment

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Innovative therapies targeting replicating and dormant Mycobacterium tuberculosis are critical for the development of more effective and shorter treatments for active and latent tuberculosis (TB). Cheminformatics studies shown that drugs endowed with anti-TB activity have the peculiarity of being more lipophilic than many other antibacterial compounds. The interaction of the lipophilic moiety with the membrane alters its stability and functional integrity due to the disruption of the proton motive force, resulting in cell death. Due to the emergence of drug resistance, there is an urgent need of novel inhibitors. As drug efflux is an important contributor to M. tuberculosis drug resistance, the identification and characterization of lipophilic efflux inhibitors may be useful for the development of new effective therapies.

We have identified and characterized a novel efflux inhibitor - UPAR-174 - with efficacy against replicating and nonreplicating M. tuberculosis. UPAR-174 is active, at nontoxic concentrations, against intracellular drug resistant bacteria combined with antibiotics. It targets cell membrane integrity through the dissipation of membrane potential and efflux inhibition and showed reduced predisposition to develop drug resistance. The lipophilic profile of UPAR-174 mirrors that of the most recent anti-TB drugs and points towards the relevance of lipophilicity in TB drug discovery.

Targeting M. tuberculosis with lipophilic efflux inhibitors, exploring their dual activity - dissipation of the proton motive force and efflux inhibition - represents an attractive strategy to prevent the emergence of multi- and extensively drug resistant TB. UPAR-174 showed to be a promising molecule for the development of new drugs and therapeutic strategies against TB and the characterization of its mechanism of action has contributed for further optimisation of the scaffold in order to increase its antimycobacterial activity and reduce toxicity. Combining biology and medicinal chemistry, a novel series of derivatives was designed and is being studied to achieve the major goal of shortening TB treatment.

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Poster 10

Urban contamination and brain degradation: the missed link?

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Background: In polluted areas the ambient air contains particulate matter, PM. Such undesired components can be diverse in size (ultrafine, fine and coarse) as well as in chemical composition. Particles with a size below 100 nm have been described to promote adverse health effects as they can easily penetrate the body of humans being accumulated in the liver, kidneys, heart, and brain with tremendous health consequences. Effects of air pollution on health have been largely studied, resulting in pathologies ranging from ophthalmologic problems to respiratory and neurological issues or even death. [1

Objectives: Here, we report on the variation of the proteomic response with time of mice brain after 1, 3, and 6 months of exposure to ultrafine particulate, UFP, matter isolated from Los Angeles basin air.

Methods: To study the effects of UFP on the brain we have used six groups of mice divided into mice breathing purified air (control group) and mice breathing air with UFP. Expose

d and control mice were sacrificed after 1, 3 and 6 months. High-Resolution Label Free-based Shot-Gun Mass Spectrometry was used to quantitatively study the proteomics changes.

Results: Proteomic changes driven by urban pollution suggest the particulate matter as a deregulator of energy metabolism, mitochondrial activity, and oxidative pathways in the mice brain

Conclusions: Overall, the proteomic analysis revealed that two main biological features are dysregulated in the rat brain after exposure to PM: the mitochondrial activity, through the activation of pathways linked to the respiratory chain, and the neuronal/astrocyte activity, causing brain changes that trigger tumorigenesis and neurodegeneration [2]

References: [1] https://ec.europa.eu/jrc/en/news/what-are-main-sources-urban-air-pollution, accessed in 30th July 2020. [2] Araújo, E. et al., STOTEN, 2019, 839-848, 687,doi:10.1016/j.scitotenv.2019.06.102

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Poster 11

The fallen of biomarkers and the rise of personalized medicine

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Background: The promises of biomarker discovery as a powerful tool to diagnostic and prognostic diseases have not been filled yet and it seems it will never be. The problem of biomarkers in disease is that many on the ones described in the last ten years have a very limited diagnostic and prognostic value. Many biomarkers respond to inflammation, infection or other physiological conditions derived from disease but cannot be linked directly to the disease itself, specially to prognostic or to treatment response. Furthermore, the phenotype of the patient plays an important role in biomarker response. However, modern mass spectrometry has brought a new world of opportunities to visualize the evolution of the patient´s proteome as the disease evolves. Thus, instead of a couple of biomarkers, the levels of tens of hundreds of proteins can be now monitored using high resolution mass spectrometry and the relations interplayed by the biochemical pathways in which such proteins are involved can be unveiled. Thus, it is easy to unravel in an individual the way hallmarks of disease follow, so the treatment can be adjusted or modify accordingly.

Objectives: To show the Medical community the latest trends in Personalised Medicine based proteomics thought the study of bladder cancer patients.

Methods: The proteome of eighteen T1 stage bladder cancer patients were deep mining and compared using bioinformatics to unveil prognostic.
Results: Prognostic is possible on base on the patient ´s levels of hallmarks of cancer.

Conclusions: Overall, the proteomic analysis revealed a large number of biochemical pathways dysregulated at a different level for patients with poor or bad outcome.

References: Precision Medicine: Role of Proteomics in Changing Clinical Management and Care, Jennifer E. Van Eyk and Michael P. Snyder. J. Proteome Res. 2019, 18, 1, 1–6

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Poster 12

Precision and Personalized Medicine Facility

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Background: The advent of High-Resolution Mass Spectrometry, the development of powerful bio-informatic tools and the high-resolution sequencing of the human genome have situated proteomics as the ultimate tool for personalized diagnosis, prognosis and assisting drug-based treatment of diseases. Currently genetic modifications can be visualized with proteomics, being not necessary the use of genetic techniques to unravel genetic-driven diseases. Furthermore, the response to drugs can be followed in such a way that drug treatment and drug dosage can be personalised.

Objectives: To show the medical community the most advanced laboratory for personalised medicine of the NOVA University and also the latest trends in personalised medicine-based proteomics thought the study of diseases.

Methods: The proteome of nasopharyngeal carcinoma patients were deep mining to unravel prognosis and diagnostics.

Results: Prognostic is possible on base on the patient ´s levels of hallmarks of cancer. The influence of virus such as Epstein-Barr and Herpes Simplex in the diseases are highlighted.

Conclusions: Overall, the proteomic analysis revealed a large number of biochemical pathways dysregulated at a different level for patients with poor or bad outcome.

References: Precision Medicine: Role of Proteomics in Changing Clinical Management and Care, Jennifer E. Van Eyk and Michael P. Snyder. J. Proteome Res. 2019, 18, 1, 1–6

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Poster 13

Optimization of a Red Nile-based real-time fluorometric assay for the study of competition between efflux pump substrates

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The AcrAB-TolC efflux pump (EP) of Escherichia coli is a major contributor to multidrug resistance (MDR) since it can export a variety of drugs, dyes and detergents. There are various methods to measure the activity of EPs in bacterial cells which can be divided, essentially, into two categories (i) those that measure efflux directly by quantifying how much substrate is pumped out; and (ii) those that measure efflux indirectly by quantifying how much substrate is accumulated inside the cell. However, these methods are not able to measure competition between EP subtracts.

To study the existence of substrate competition, we optimize a real-time accumulation assay in a high-throughput format using 96-well microplates for the Synergy HT microplate reader, using Red Nile, a substrate of AcrAB-TolC, as fluorophore/competitor and E. coli as a model. The MICs of each substrate and efflux inhibitors was determined using a 96-well microplate by two-fold serial microdilution of each compound in Mueller-Hinton broth. We used the protonophore carbonyl cyanide-m-chlorophenylhydrazone (CCCP) to de-energize the cells. After the addition of glucose as energy source, efflux was restarted after 100 sec. All substrates were tested using the following range of concentrations: MIC to 1/8 MIC.

E. coli strains studied were ATCC25922 and AG100 K-12, both with functional AcrAB-TolC EP; AG100A, with the AcrAB-TolC inactivated; and AG100Tet, with AcrAB-TolC overexpressed. The substrates tested were acriflavine, chloramphenicol, ciprofloxacin, deoxycholic acid, doxorubicin, doxycycline, erythromycin, gentamicin, minocycline, oxacillin, safranin, tetracycline, tetraphenylphosphonium bromide; and the efflux inhibitors were chlorpromazine and phe-arg-β-naphthylamide.

The results we have so far, allowed us to show that, of all substrates tested, only safranin competes with Nile Red efflux, in a dose dependent manner. In this work we have validated a new fluorescent molecule to quantify efflux activity by binding to a different site on AcrAB. The identification of drugs that inhibit antibiotic efflux through competition for the active site of the pump is a value tool for the study of EPs activity and discovery of new compounds.

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Poster 14

Active efflux systems as a survival mechanism in persister cells of Escherichia coli clinical strains

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Bacterial persisters are an important source of chronic and recurrent infections. Recent studies hypothesized that persisters employ a defence mechanism via enhanced efflux activity to expel toxic compounds allowing persister formation before entering dormancy. We investigated the contribution of efflux activity to the emergence of Escherichia coli persisters in pan-susceptible clinical isolates.

E. coli ATCC25922 and five clinical strains were exposed to rifampicin to induce tolerance and persister cells selected with ampicillin. The presence of active efflux systems was studied by MIC determination in the presence of efflux inhibitors (EI), real-time fluorometry using ethidium bromide as fluorophore for efflux activity evaluation and synergism assays with EI. As controls we used three E. coli isogenic strains that differ in the activity of their main efflux system, the AcrAB-TolC: wild-type AG100 with a functional efflux pump (EP), AG100A with an inactivated EP and AG100tet with EP overexpression.

The results showed that E. coli persisters can be induced with rifampicin, displaying a typical biphasic killing curve. This data confirms the development of tolerant persister phenotypes under drug pressure and that persisters can be selected with ampicillin after induction with rifampicin. MIC assays (18h – long-term response) showed that the persister cells were as susceptible as the parent strains. However, the real-time fluorometry assays (30min – short-term response) revealed the existence of increased efflux activity in persisters, which could be inhibited by EIs.

These results show the importance of active efflux systems as a survival mechanism in persister cells of E. coli clinical strains, which can be inhibited in the presence of EIs. This work supports the hypothesis that the activity of EPs allows the maintenance of an antibiotic tolerant population in a sub-optimal treated patient from which genetically resistant mutants emerge. The use of compounds that can inhibit EPs in persister cells in clinical therapy, optimizing the effect of antibiotics, will increase the effectiveness of currently used therapies for the treatment of these infections.

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Poster 15

Tackling β-lactam resistance in Escherichia coli – the role of efflux pumps in β-lactamase producing strains

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β-lactam resistance is a major threat to global health especially in the clinical setting. Antibiotic resistance rates in Escherichia coli are rising worldwide representing a worrying issue to medicine. E. coli develops resistance to β-lactams mainly by β-lactamase production. The role of efflux to β-lactam resistance remains unclarified. Here, we aimed to understand the role of efflux on β-lactam resistance in E. coli.

27 clinical isolates were typed by ERIC-PCR and the phylogenetic groups determined using the Clermont method. Antibiotic susceptibility profiles were determined by disc diffusion. Isolates were screened for genes encoding β-lactamases by PCR. Efflux activity was studied by synergism assays with efflux inhibitors (EIs) and ethidium bromide (EtBr) real-time fluorometry assays. As controls, we used the ATCC25922 and three E. coli isogenic strains with well-characterized efflux activity: wild-type AG100, AcrAB pump-deficient AG100A, AcrAB overexpressing AG100tet.

Strain typing revealed diversity of ERIC patterns among the isolates. 18 strains belong to group B2 (virulent extra-intestinal), four belong to group B1 (commensal) and seven strains could not be differentiated. 13 isolates showed an MDR phenotype and 11 were non-MDR. All clinical strains were resistant to β-lactams, but only one was resistant to extended-spectrum cephalosporins. MDR strains presented resistance to quinolones, chloramphenicol, tetracyclines and/or aminoglycosides and all were susceptible to carbapenems and tigecycline. β-lactamase production was detected in all strains and included the presence of TEM in combination with OXA, SHV or CTX-M. The presence of mutations in the AmpC promoter region was screened by DNA sequencing and revealed mutations in some isolates. The results showed that EIs act synergistically with antibiotics and EtBr, reducing MIC values which may be correlated with the inhibition of their efflux. The real-time efflux assays demonstrate that increased efflux activity could be inhibited by EIs.

Our results demonstrated the importance of efflux systems to β-lactam resistance and the use of EIs should be considered for a future therapeutic approach to tackle β-lactam resistance.

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Poster 16

Using genomics to track the contribution of efflux pumps and carbapenemases to carbapenem resistance in Acinetobacter baumannii

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Drug resistant Acinetobacter baumannii (Ab) is a public health threat requiring urgent and aggressive measures. Moreover, carbapenemase-producing Ab can disseminate mobile resistance genes to other bacteria increasing the risk of resistance. We performed an in vitro and bioinformatic analysis to study the mechanisms contributing to CARB resistance in 72 clinical isolates of Ab.
Carbapenemases were screened by PCR. Efflux activity was studied by synergism assays between CARB, ethidium bromide (EtBr) and efflux inhibitors (EIs), real-time fluorometry and expression analysis of efflux pump (EP) genes in response to imipenem. Search of EP candidate proteins and their orthologous was done using NCBI and KEGG. BLASTP was done to compare predicted proteins and quantify similarity between amino acid sequences. Genomic arrangement of orthologue genes was analysed using SyntTax. Subsystem functional categorization of predicted CDS and multi-genome comparison was done using SEED.
All strains were multidrug resistant and resistant to CARB harbouring OXA-23 or OXA-24 carbapenemases. Synergistic interactions between antimicrobials and EIs were noted as well as the presence of active efflux. EPs were found overexpressed with AbeS, a small multidrug resistant (SMR) EP, showing the highest levels of expression. Genomic analysis was performed and were identified 6 SMR EPs: 3 chromosomally encoded (AbeS, EmrE, SugE) and 3 encoded in plasmids or class 1 integrons (QacF, QacE, QacEΔ1).
We show that efflux contributes to CARB resistance in Ab. The presence of several SMR EPs in Ab genome might explain its high predisposition to survive in presence of antimicrobials. This preliminary in silico approach showed helpful to predict important physiological responses aiming the development of new antimicrobials against drug resistant Ab.

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Poster 17

Haem biosynthesis in Campylobacter jejuni?

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Campylobacter jejuni is the cause of 1/4 of diarrhoeal diseases in the world. Thus, is considered to be the major cause of gastroenteritis worldwide. Although these infections are mostly not life threatening, it can be fatal in high risk groups. The increasing amount of infections due to C. jejuni demands a better understanding of the molecular mechanisms allowing its survival within the host. Furthermore, C. jejuni secretes toxins, like cytolethal distending toxin (CDT) that cause DNA damage, which may contribute to the initiation of colorectal cancer and formation of a tumor microenvironment (1, 2). Still, the molecular mechanisms underlying C. jejuni colonization remains poorly understood. This includes the role of haem biosynthesis that plays a crucial role in bacterial pathogens, as their survival within the host rely on essential haem-binding proteins. Indeed, haem is responsible for the function of several essential cellular processes such as, respiration, signaling, gas sensing, microRNA processing and cellular differentiation. Pathogens can obtain haem either by acquiring it from the host or by synthesizing it. Hence, most prokaryotes are able to synthesize haem endogenously via specific Haem Synthesis Pathways (HSP). All known HSP begin with the universal tetrapyrrole precursor -aminolaevulinic acid and requires a cascade of enzymes to produce haem. The first pathway discovered, named protoporphyrin dependent pathway (PPD), requires at least eight enzymes to form haem. Recently, our group participated in the discovery of two other distinct pathways, named sirohaem and coproporphyrin dependent pathway, which occur in sulfate-reducing bacteria and Gram-positive pathogens, respectively (3–5). In this work, we study C. jejuni's haem biosynthesis pathway and its role in pathogenicity. We will present our results on the biochemical characterization of the haem biosynthesis enzymes and their in vivo function. Altogether, the data will uncover the role of HSP pathway in C. jejuni infection process. **1. He Z et al. Gut. 68(2):289. (2019) 2. Brauner A et al. Scandinavian Journal of Gastroenterology. 45(7–8):893. (2010) 3. Bali S et al. Proc. Natl. Acad. Sci. U.S.A. 108(45):18260. (2011) 4. Lobo SAL et al. Mol. Microbiol. 97(3):472. (2015) 5. Videira MAM et al. Mol. Microbiol. 109(3):385. (2018**

Poster 18

Flowsheet Optimization for the Isolation of Recombinant STEAP1 from Komagataella pastoris Bioreactor Cultures

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The Six-Transmembrane Epithelial Antigen of the Prostate (STEAP) family of proteins share a six-transmembrane domain and intracellular N- and C- termini, although differ in cellular functions and expression patterns. Based on amino-acid sequence, transmembrane topology, and cellular localization, it was suggested that STEAP1 could act as transporter, being involved in cellular communication and in stimulation of cell growth by increasing the levels of ROS. Also, STEAP1 seems to contribute to maintaining metal homeostasis, oxidative stress response, cell-cell communication, proliferation, invasion and apoptosis. Considering that no expression is detected in normal cells but it is mainly over-expressed in the cell membrane of prostate cancer (PCa) cells, STEAP1 has been pointed as an attractive biomarker and immunotherapeutic target. In fact, several studies have shown that over-expression of STEAP1 enhances cancer cell proliferation and contributes to tumour development and aggressiveness. Although STEAP1 is linked to PCa, the determination of a high-resolution structure of the protein, as well as the mechanisms underlying its function remains elusive. Our goal was to isolate the STEAP1 protein in a single fraction with a high degree of purity. We evaluated the fraction capacity of typical hydrophobic matrices (Octyl, Phenyl and Butyl-Sepharose) on crude lysates obtained from Komagataella pastoris X-33 Mut+ mini-bioreactor cultures. Since the ionic strength affects the interaction between the hydrophobic domains of STEAP1 and chromatographic matrices, we thoroughly analysed the effect of salt concentration upon protein elution. Interestingly, STEAP1 was fully retained onto Butyl-Sepharose matrix, using only 50 mM (NH4)2SO4 as binding buffer. In less hydrophobic matrices this interaction was partial (Phenyl) or inexistent (Octyl). The Butyl support also provided a selective elution of STEAP1 throughout a linear gradient from 10 mM Tris to H2O. Both buffers were supplemented with 0.1 % SDS to mimic the protein's native environment. Size exclusion chromatography allowed to obtain a monodisperse sample of STEAP1. In this work, we established a procedure for the purification of recombinant STEAP1 with a considerable purification yield, using a combination of traditional hydrophobic techniques. These results will prompt us to gather a detailed functional and structural characterization of STEAP1.

Poster 19

One piece more in the puzzle of Plasmodium simium and Plasmodium vivax identity: detection of P. vivax in a liver sample of a howler-monkey

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Framework: Malaria is the most common parasitic disease worldwide and global efforts aim to reduce the prevalence and mortality in 90% by 2030. Factors are hampering the disease control, such as human asymptomatic carriers and animal reservoirs. The limited identification of asymptomatic cases hinders their treatment, impairing the disease control. Where simians might participate in the life cycle of the parasite the risk of ongoing transmission also might increase. In Brazil, 1% of malaria cases refers to their occurrence in the extra-Amazonian region. Although interrupted in the 1960s, it has persisted in areas of dense Atlantic Forest as an endemic disease with low frequency of cases, many of them asymptomatic. Outside Amazonia, Espírito Santo State (ES) register the highest number of cases nationwide. The chronological and spatial distance between the cases does not fit to the classical malaria cycle, which raises the suspicion of a sylvatic reservoir. There, simian and human malaria occurs at the same places. Parasites are called Plasmodium simium when infecting simian hosts despite the evidence pointing at genetic identity with its human counterpart Plasmodium vivax. Recently, the identification of genome markers to differentiate P. vivax and P. simium in isolates from Atlantic Forest led to the development of a PCR-RFLP protocol to monitor the infections in some Brazilian states. Until now, none of the simian parasites tested by this protocol are reported as P. vivax and human cases found with P. simium were considered zoonotic infections. Objectives: To verify the effectiveness of the PCR-RFLP protocol in Plasmodium spp. DNA from mosquitoes and simian collected at ES. Results: One fragment of a simian liver was positive for P. vivax, in contrast to the expectation based on the previous results of the protocol. In the mosquitoes' samples, both forms of the parasite were identified. Conclusion: These results suggests that the published PCR-RFLP protocol are not able to elucidate the dynamics of Plasmodium spp. circulation in the Atlantic Forest of ES. New methodology and markers are needed for a deeper understanding of the transmission cycle of this singular endemic disease.

Poster 20

Personalised medicine in bladder cancer as never seen before

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Background
Bladder cancer (BCa) is the most common cancer of the urinary tract and is the sixth more incident cancer worldwide, in the male gender [1]. A patient who is diagnosed with BCa needs to be continuously followed up, due to the high risk of recurrence and progression leading to a high economic burden disease [2,3].

Objective
In this study, we aim to develop a non-invasive follow-up method for T1 stage BCa patients. We choose to focus on T1 bladder cancer patients as this group requires a more severe follow-up since there is a subgroup of T1 stage BCa with bad prognosis outcome that has a higher aggressiveness and can rapidly progress to a higher stage.

Methods
We used urine as a source to identify a potential protein that can act as biomarkers with value to access T1 bladder cancer. Urinary proteome was processed using the methodology filter-aided sample preparation and further analysed by high-resolution mass spectrometry. The protein identification and quantification data were further achieved by using bioinformatic analysis in MaxQuant V1.6.0.16 and Perseus 1.6.5.0.

Results
Different proteins were identified as potential candidate biomarkers. From all the potential proteins, a ratio of two specific proteins showed up to have a high level of prognosis value that can potentially predict the outcome of patients who have T1 BCa stage.

Conclusion:
Our results suggest that the urinary proteome can be used as a tool to follow-up the outcome of T1 stage BCa patients. Furthermore, the proteomics study herein showed shows that the levels of those two proteins can be used as a tool in BC prognosis.

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Poster 21

Profile of potential human stress biomarkers in sweat associated with physical training

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Keywords: Biomarkers, Stress, Physical training, Sweat, LC-MSMS

Background Stress, both physical as psychological, that has been related to several diseases and discordant social behaviour. Stress biomarker molecules detection is an important field of research since has obvious implications in the potential development of new methods and/or devices with interest in medicine. Less invasive methods are also being pursued for continuous health monitoring. In the current study, sweat is the target fluid to be studied aiming the identification of potential stress biomarkers that may facilitate the in-situ detection and monitoring of stress-related disease states.

Methods This study has been performed for a global identification analysis on pooled sweat samples collected from ten healthy individuals. An analytical method to attain a screening profile of sweat composition in only one LC-MSMS analytical step was developed. One day prior the match, the healthy volunteers did not shave or took any medications nor use any deodorants, face or body creams or perfume.

Results From the identified molecules some were already described stress related biomarkers, (e.g. cortisol, epinephrine). In general were identified more 26 potential identified biomarkers, mostly of them neurotransmitters (NTs) and its metabolites amongst other molecules.

Conclusions The composition profile of pooled sweat samples provided the identification of 26 potential biomarkers by LC-MSMS. This identification is an important achievement for further studies and selection of most important biomarkers in sweat, not only for stress, but also for other possible conditions/pathologies or early diagnosis.

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Poster 22

Infodemiology of COVID-19: study of web searches and preventive measures taken during the pandemic in Angola

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Background Since the first cases of COVID-19 in China, numerous scientific, informational articles have been edited in various formats about the disease, resulting in a global infodemic. In Angola, many searches were done to increase health literacy for COVID-19. The web search engine enabled this frantic search. Objective: the aim of this study is to investigate internet search behaviors in Google trends related to COVID-19 and relate preventive measures adopted during the COVID-19 epidemic in Angola. Methods: this is an infodemiology study, in which five keywords were used as an information search strategy: "Covid"; "Coronavirus", "Corona", "quarantine" and "calamity", having as main inclusion criteria words that generate at least 30% of the peak of popularity in Google Trends (GT) searches in Angola and in the world, in English and Portuguese. This search was limited to 1 January 2020 to 14 September 2020. The official website of the Inter-ministries Commission of Angola was used to find out the social measures and recommendations defined by the Angolan government to prevent COVID-19 Results: The most intense search on COVID-19 in Angola occurred during the weeks of 13-26 March 2020. During this period, Angola had 4 cases of covid-19, and the government approved a provisional presidential decree which prohibited the free movement of people and non-essential goods, promoted frequent hand hygiene, decreed a period of 14 days of quarantine for persons coming from foreign countries, etc. The keywords most used to search for information about COVID-19 in Angola were "Covid + 19", "Coronavirus", "prevention + coronavirus", "treatment + coronavirus" and "Vaccine + Coronavirus", in all categories. "Coronavirus" was the most used term to search for COVID-19 information in Angola and worldwide. The province of Angola where the most sought after information online about COVID-19 was Lunda Norte. Conclusions: The search for information in Angola began at the end of January 2020, and peaked in mid-March 2020, probably coinciding with the World Health Organization declarations of Public Health Emergency of International Concern on 30 January 2020 and, the pandemic declaration on 11 March 2020. People were more concerned with getting to know the novel coronavirus, collecting information about the disease, the vaccine, preventive measures and treatment for COVID-19.

Poster 23

What are the barriers faced by migrant women to participate cervical cancer screening? A scoping review of European evidence

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Abstract

Background: Migrant women are a vulnerable group for cervical cancer. Screening can reduce cervical cancer mortality, but migrants participate less in cervical cancer screening (CCS) compared to native women. This study aims to provide an overview of the main barriers associated with the participation in CCS among migrant women in Europe.

Methods: A scoping review of original research articles was conducted in electronic peer-reviewed databases. Quantitative and qualitative studies on factors related to the participation of migrants in CCS in EU/EFTA were retrieved and selected for data extraction. Barriers found were grouped in sociodemographic, healthcare-system, psychological, migration, knowledge, language, and cultural factors.

Results: 20 out of 96 articles were included. Language barriers, difficulties in accessing healthcare services, lack of information about CCS, and emotional responses to CCS were the most reported barriers. Women's health is a taboo in some cultures which raises some specific issues in participating in CCS, such as being seen by a male healthcare professional or suffering from social stigma. Sociodemographic factors, such as age, education, employment and marital status, often showed conflicting results.

Conclusions: To increase migrants' women participation in CCS, it is important to target not only general barriers to access healthcare services faced by them, but also develop strategies to reduce sociocultural barriers related to reproductive health, and specifically CCS. Healthcare providers working with CCS should be aware of cultural differences about sexuality when addressing these issues with migrant women and may have an important role educating them and eliminating stigma associated with CCS. Women should have the option of choosing to be seen by a female healthcare professional when attending CCS. Providing translated information can also help promoting CCS in these communities. These findings can help improving CCS programs to increase migrant women's participation and enhance their overall health.

Poster 24

Optimizing gene therapy vectors for Retinitis Pigmentosa

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Background: Retinitis Pigmentosa (RP) is the most common form of inherited retinal degeneration. It is characterized by the degeneration of photoreceptors, leading to nyctalopia and, in advanced stages, daylight vision impairment. It is caused by mutations in several different genes; hence gene therapy is a promising therapeutic approach.
Amongst the vectors used for gene therapy, non-viral vectors have great potential; however optimisation is still needed for higher gene transfer efficiency. Our previous work showed that entering the nucleus was the main obstacle for nonviral vectors, as retinal cells are post-mitotic. Therefore, we aim to increase the efficiency of our nonviral vectors by conjugating nuclear localization signals (NLSs), which are amino acid sequences responsible for directing molecules to the nucleus.

Methods: To test our hypothesis, we have generated a plasmid expressing the gene of the β subunit of PDE (mutated in a subset of RP), combined it with our optimised nonviral vector [poly(2-aminoethyl methacrylate) (PAEMA) conjugated with NLSs] and further tested this strategy in vitro using a human retinal pigment epithelial cell line (D407) and in a mouse model of this form of RP, the rd10 mice.

Results: We have found that our nonviral vector can be efficiently conjugated with NLSs, to incorporate the therapeutic plasmid and to protect it from degradation. Furthermore, in retinal pigment epithelial cells, used to test the polyplexes for their transfection efficiency both qualitatively (fluorescence microscopy) and quantitatively (flow cytometry and RT-qPCR) have shown that the optimised nonviral vector is capable of promoting the expression of the gene of interest. Lastly, the intravitreous injection of the nonviral vectors in the retina of a Retinitis Pigmentosa mouse model (rd10 mice) has so far shown this vector to be biocompatible and non-inflammatory in the mouse retina.

Conclusion: Overall, these results demonstrate the potential of PAEMA nonviral vectors for retinal gene therapy.

Poster 25

New Insights into Immunological Involvement in Congenital Disorders of Glycosylation (CDG) from a People-Centric Approach

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Background: Abnormal glycosylation has been identified as an underlying cause of various human diseases, such as in cancer and in congenital disorders of glycosylation (CDG). CDG are rare diseases with variable phenotypes and severity. Immunological involvement remains poorly understood in CDG, mainly due to lack of robust data.
Objective: To characterize immune-related manifestations' prevalence, relevance, and quality-of-life (QoL) impact by harvesting the direct knowledge and experience of CDG patients and caregivers.
Methods: We developed electronic questionnaires (e-questionnaires) targeting (1) CDG patients and (2) the general "healthy" population. The e-questionnaires were divided into several sub-sections, including infections, allergies, autoimmune diseases, vaccination and QoL impact of immunological manifestations.
Results: We included 209 CDG patients/caregivers and 349 healthy participants. The most prevalent CDG was PMM2-CDG (n = 122/209). About half of PMM2-CDG patients (n = 65/122) described relevant infections with a noteworthy occurrence of gastrointestinal tract (GI) infections (63.1%, n = 41/65). Infection burden and QoL impact were shown to be correlated with more severe clinical phenotypes and with a set of relevant non-immune PMM2-CDG signs. Autoimmune diseases had a marginal presence in PMM2-CDG (2.5%, n = 3/122), all being GI-related. Allergy prevalence was also low in PMM2-CDG (33%, n = 41/122) except for food allergies (26.8%, n = 11/41 of PMM2-CDG and 10.8%, n = 17/158 of controls). High vaccination compliance with greater perceived ineffectiveness (28.3%, n = 17/60) and more severe adverse reactions were described in PMM2-CDG.
Conclusion: This people-centric approach not only confirmed literature findings, but created new insights into immunological involvement in CDG, namely by highlighting the possible link between the immune and GI systems in PMM2-CDG. Our results emphasized the importance of patient/caregiver knowledge and raised several red flags about immunological management.

Poster 26

Insights on colistin-heteroresistant Acinetobacter baumannii towards the development of effective therapies

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Colistin is regarded as the last option against carbapenemase-producing Acinetobacter baumannii (Ab). We studied the mechanisms of resistance to colistin in eight Ab strains isolated from fatal bloodstream infections in elderly patients acquired during therapy.

Heteroresistance to colistin was studied using E-test, broth microdilution and Levine-agar assay; ERIC-PCR and MLST for genotyping; and drug resistance target mutation analysis. Biofilm formation and viability was determined by CFU´s determination and the resazurin method, respectively. Drug efflux was studied by MIC determination for colistin in the presence of efflux inhibitors (EIs), ethidium bromide (EtBr) real-time fluorometry and analysis of mRNA transcriptional levels of efflux pump (EP) genes after exposure to colistin.

All strains harbour mutations in the operons LpxACD and PmrAB associated with colistin resistance. MIC reductions ranging from 256 to 8-fold of colistin in the presence of the EIs were obtained. Efflux assays demonstrated increased efflux activity in these strains which could be inhibited by the EIs. The EP genes adeB, adeJ, adeG, craA, amvA, abeS and abeM were found overexpressed in response to colistin exposure. All strains displayed heteroresistance to colistin. We observed three strain-variants exhibiting different colony morphologies, growth rates, and susceptibilities to colistin. The colistin sub-population showed increased capacity to form biofilms.

We put in evidence the high versatility by which this bacterium manages to survive treatment. Understanding the mechanisms behind the development of colistin resistance will have a direct impact on the develop of more effective therapies, as the use of EIs, and will give additional information on how to implement appropriate approaches for its detection.

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Poster 27

Towards mass spectrometry-based pathology

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Background: Renal cell carcinoma (RCC) is a heterogeneous disease comprising several subtypes such as clear cell (ccRCC), papillary (pRCC), chromophobe (chRCC), and renal oncocytoma (RO). Although the different histologic RCCs subtypes can be classified on the basis of morphologic and immunohistologic characteristics, some overlapped features are common between subtypes, making diagnosis difficult.
Objectives: In this work, we propose to use for the first time the total proteomic approach (TPA) to identify an effective panel of proteins and their concentrations ranges to diagnose and characterize diverse RCC subtypes
Methods: Human renal tissue biopsies were obtained from patients diagnosed with ccRCC (n=7), pRCC (n=5) chRCC (n=5) and RO (n=5). Normal adjacent tissues (NAT) specimens (n=5) were used as control. The proteomes extracted from renal biopsies were digested with trypsin and analyzed by nanoLC-MS/MS. Label free quantitative analysis was performed by comparison between protein abundances of tumors and NAT specimens. TPA approach was used to transform the LFQ values of proteins.
Results: The analysis of the proteome of 27 RCC samples has allowed us to identify a total of 850 differentially expressed proteins between groups. Next, TPA-based concentrations have revealed a 24 panel, composed by the six proteins with the highest significant differential expression for each tumor subtype, which can be used to classify the biopsies.
Conclusions: A top 24 panel, based on TPA concentration values, able to discriminate each subtype is proposed for clinical diagnosis. In a near future, we envision the TPA based pathology as the next step in solid biopsy-based cancer diagnosis and prognosis.
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Poster 28

Enhanced antimicrobial activity of Ionic liquids and organic salts derived from antibiotics

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Chemical enhancement of standard antibiotics is a very promising way to potentiate the antimicrobial activity of such drugs, including to tackle resistant strains. Over the last years, our group has focused on the preparation of Pharmaceutical Ionic Liquids and Organic Salts based on β-lactam and fluoroquinolone antibiotics to enhance their antimicrobial activities as well as increase bioavailability and eliminate polymorphism.
Herein, we present the preparation of organic salts and ionic liquids (OSILs) from four well-known fluoroquinolone (FQ) and β-lactam antibiotics: ciprofloxacin [CIP], norfloxacin [NOR], penicillin G [PEN] and amoxicillin [AMX] via an ammonia buffered neutralization of the antibiotics combined with several organic cation hydroxides.
The obtained in vitro minimum inhibitory concentration (MIC) data of the prepared hydrolysed-β-lactam-OSILs showed a relative decrease of the inhibitory concentrations (RDIC) of 100 for [C2OHMIM][seco-Pen] against sensitive S. aureus ATCC25923 and, most strikingly, higher than 1000 for [C16Pyr][seco-Amx] against MRSA ATCC 43300.
The prepared FQ-OSILs are not toxic to healthy cell lines and displayed up to 1416-fold higher solubility in water than the original drugs. In general, the antimicrobial activity against K. pneumoniae is particularly enhanced for the ciprofloxacin-based OSILs, with RDICs as high as 20, while activity against S. aureus and the commensal B. subtilis strain was often reduced.
Hence, depending on the cation–drug combination, broad-spectrum or strain-specific antibiotic salts have been achieved, potentially leading to the future development of highly bioavailable and safe antimicrobial ionic formulations as well as great change of the pharmaceutical activity of a drug, including giving rise to potent formulations of antibiotics against resistant bacteria strains.

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