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LOUIS PASTEUR

November 29–30, 2022

WARSAW, POLAND



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Control of the saprophyte-to-pathogen transition in *Listeria*: mechanism and clinical implications

O005

José A. Vázquez-Boland[1]

[1] Edinburgh Medical School (Biomedical Sciences-Infection Medicine), University of Edinburgh, United Kingdom

Listeria monocytogenes virulence is controlled by PrfA, an allosterically regulated transcription factor. PrfA is essential for both coordinating the activation of the listerial virulence programme during infection and preventing wasteful production of virulence factors outside the host, thus maximizing the pathogen's transmission fitness. I will review how *L. monocytogenes* PrfA senses the transition from saprophyte to pathogen via the peptide composition of the bacterial habitat and discuss new data about the structural mechanism of promiscuous PrfA inhibition by nutritional peptides. The presentation will also illustrate how basic research into PrfA regulation and the mechanisms of *Listeria* intracellular parasitism can translate to direct clinical applications in the treatment of listeriosis.

Interplay between stress and drug resistance in pathogenicity of *Cryptococcus neoformans*

O006

Łukasz Kozubowski[1]

[1] Department of Genetics and Biochemistry, Clemson University, Clemson, SC, USA

Cryptococcus neoformans is a pathogenic yeast-like fungus that causes lethal meningitis in immunocompromised patients. One of the challenges in treating cryptococcosis, especially in the geographical areas where the infection is most prevalent, is the development of resistance to azole antifungals. Previous studies have demonstrated that resistance to the azole drug fluconazole (FLC) results from elevated copies of critical resistance genes in aneuploid cells. However, how aneuploidy is formed in the presence of FLC remains unclear. Our studies explore the effects of FLC on *C. neoformans* at a cellular and population level. We find that FLC leads to pleiotropic defects, affecting synchronization between growth and cell cycle progression. Our data also suggest that FLC causes DNA damage through forming complexes with metals and generating reactive oxygen species, which may contribute to development of resistance. At a population level, we characterize factors that influence phenotypic heterogeneity within *C. neoformans* population and how they affect the response to FLC. We model how variable reaction to FLC within the population changes depending on FLC concentration and factors that influence the rate of cellular growth. Our findings point to a multifactorial nature of the evolution of the resistance to FLC.

virulent mycobacteria into a clonal complex of highly virulent pathogens of mammalian hosts. It is discussed how this evolution is linked to the gain of selected features as well as the loss of genetic material, both representing crucial factors that may influence the survival and persistence of tubercle bacilli in given hosts.

Mechanisms of solvent tolerance in *Pseudomonas* O028

Juan-Luis Ramos[1]*, Ana Garcia-Franco[1], Patricia Godoy[2], Estrella Duque[2]

[1] Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain
[2] Department of Environmental Protection, Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain

A number of microorganisms have the ability to thrive in the presence of a range of toxic solvents. Tolerance to these chemicals is a multifactorial process, meaning that bacterial cells use a set of physiological and gene expression changes to overcome the damage imparted by these chemicals. I will focus on issues related to tolerance to aromatic hydrocarbons and in *Pseudomonas*. *Pseudomonas putida* strains contain a circular chromosome of approximately 6 Mbp which encodes about 5300 genes. A combination of physiological and biochemical assays, a genome-wide collection of mutants and several omics approaches have provided useful information to help identify functions involved in solvent tolerance in *P. putida*. The solvent response involves fine-tuning of lipid fluidity to adjust membrane functions including impermeabilization, activation of a general stress-response system, increased energy generation and induction of specific efflux pumps that extrude solvents to the medium. These responses are modulated at the transcriptional level by local and global regulators as well as by a number of sRNAs whose levels fluctuate with the presence of solvents in the environment. Taken as a whole, these regulatory inputs orchestrate the complex network of metabolic responses observed after solvent addition.
Funded by: Ministerio de Ciencia e innovación RTI2018-094370-B-I00; European Commission: Grant agreement 862695 and 101070045; Junta de Andalucía: PAIDIP20_00049

P029 Drug resistance assessment tools evaluating positively selected amino acid sites in proteins of *Mycobacterium tuberculosis*

Antonia Saktiawati[1,2*], Agnieszka Olejniczak[1], Daria Zygała-Pytlos[1], Jarosław Dziadek[1], Alina Minias[1]

[1] Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland
[2] Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

Amino acid sites with increased quantity of nonsynonymous substitutions observable across the population show signatures of positive selection. In bacteria living under antibiotic pressure, positive selection might signal sites involved in the generation of drug resistance. We estimated the fraction of positively selected sites possibly generated through drug resistance in drug target proteins in the model of the bacterium *Mycobacterium tuberculosis*. We calculated the level of selection per each amino acid site of twenty drug-resistance-associated proteins of 3978 clinical strains of *M. tuberculosis*, and we compared our list with two bioinformatic databases commonly used to establish drug resistance – TB Profiler and MTBSeq. We found that both programs labeled roughly 40% of positively selected sites as associated with drug resistance. We conclude that the generation of drug resistance explains the presence of at least one-third of amino acid positions under positive selection in *M. tuberculosis* protein drug targets. We suggest that the inclusion of observation of evolutionary patterns might facilitate prioritizing particular mutations for further research and observation, eventually leading to better drug resistance diagnosis, better treatment outcomes, and the reduction of costs to control tuberculosis.

P030 Further insights into the genomic panorama of the *Mycobacterium kansasii* complex

Edson Machado[1], Sidra Vasconcellos[1], Lia Gomes[1], Marcos Catanho[2], Jesus Ramos[3], Luciana De Carvalho[3], Telma Goldenderg[3], Margareth Dalcomo[3], Paulo Redner[3], Paulo Caldas[3], Cristina Lourenço[4], Elena Lasunskaia[5], Vinicius Mussi[5], Lizania Spinassé[6], Solange Vinhas[6], Leen Rigouts[7], Roeland Janssen[7], Sari Cogneau[7], Pim De Rijk[7], Christian Utpatel[8], Jarmila Kaustova[9], Tridia Van der Laan[10], Han De Neeling[10], Nalin Rastogi[11], Klavdia Levina[12], Ndivhu Makhado[13], Manca Žolnir[14], Vera Jankovic[15], Jacobus De Waard[16], Maria Sisco[16], Dick Van Soolingen[10], Stefan Nieman[8], Bouke De Jong[7], Conor Meehan[17], Philip Suffys[1*]

[1] Laboratório de Biologia Molecular Aplicada a Micobactérias, Instituto Oswaldo Cruz, Fiocruz, Brazil
[2] Laboratório de Genética Molecular de Microrganismos, Instituto Oswaldo Cruz, Fiocruz, Brazil
[3] Laboratório de Referência Nacional para Tuberculose, Centro de Referência Professor Hélio Fraga, Escola Nacional de Saúde Pública, Fiocruz, Brazil
[4] Laboratório de Bacteriologia e Bioensaios, Instituto Nacional de Infectologia, Fiocruz, Brazil
[5] Laboratório de Biologia do Reconhecer, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Brazil
[6] Núcleo de Doenças Infecciosas, Universidade Federal do Espírito Santo, Brazil
[7] Unit of Mycobacteriology, Institute of Tropical Medicine, Belgium
[8] Molecular and Experimental Mycobacteriology, Research Center Borstel, Germany
[9] Department of Diagnostic Mycobacterioses, Regional Institute of Public Health, Ostrava, Czech Republic
[10] National Institute for Public Health and the Environment, The Netherlands

and Gram-negative bacteria. M23 peptidases have various functions starting with autolytic role crucial during cell expansion and division to elimination of other bacteria residing in the same ecological niche.
Our research is focused on the biochemical and structural characterization of M23 peptidases in the context of their structure-function relationship and unique antimicrobial potential against drug-resistant pathogens. We modulate and engineer their features to develop perfect antibacterial weapons, so called enzybiotics (therapeutic enzyme-based antimicrobials).
We have performed *in silico* analysis of more than 90,000 protein sequences containing conserved M23 motifs taking into consideration, among others, their modular architecture and surface charge. Based on this analysis, we created a platform for designing enzybiotics composed of a M23 catalytic domain accompanied by various cell wall binding domains. We selected the chimeric enzymes to have desired features in terms of activity, selectivity, safety and stability in a broad range of environmental conditions.

Investigation of the protective effect of monoclonal antibodies against poly-N-acetyl glucosamine (PNAG), a superficial polysaccharide, as an alternative to antibiotic-containing eye drops for the prevention of neonatal eye infections caused by *Neisseria gonorrhoeae* P057

Cagla Bozkurt Guzel[1*], Ozlem Oyardi[2], Tanweer Zaidi[2], Gerald Pier[2]

[1] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Istanbul, Turkey
[2] Department of Medicine, Harvard Medical School, Harvard University, USA

Neisseria gonorrhoeae can cause eye infections and permanent blindness, especially in newborns. Here, we aimed to develop novel treatments for the prevention of these infections. Strains collected from patients with *N. gonorrhoeae* eye infections were obtained from investigators at the Harvard Medical School, Brigham and Women's Hospital. They were studied for their susceptibility to serum bactericidal (SBA) killing by a monoclonal antibody labeled F598, specific for the conserved microbial surface polysaccharide, poly-*N*-acetyl glucosamine. In the SBA, killing of the strains was evaluated using various concentrations of the antibody, the target bacterial suspension and an antibody-depleted human complement source. Monoclonal antibody (MAb) F429, was used as negative control. Protective efficacy was evaluated in a mouse model of conjunctivitis. Seven to eight week old BALB/c mice were infected with strains and doses of *N. gonorrhoeae* to establish the model of infection. Pathology scores were quantified, using a scale of 0 (no pathology) to 4 (rupture of the ocular surface). After 36–48 hours the eyes were recovered, homogenized and the bacterial levels determined. Studies were then carried out to investigate the effect of MAb F598. For this, MAb F598 was given IP 8 hours after the infection along with applied topically at 8, 24 and 30 hours after the infection. As a result, it was determined that MAb F598 mediated potent SBA activity and use of the antibody in infected mice both decreased the pathology scores and the bacterial levels. These findings indicate a potential strategy for treatment and possibly prevention of gonococcal conjunctivitis.

P068 Comparative effectiveness of consecutive alternating administration of antivirals in Coxsackievirus B3 infection in mice

Adelina Stoyanova[1*], Simeon Galabov[2], Nikoleta Hristova[1], Stefan Philipov[3], Gerhard Pürstinger[4], Georgi Dobrikov[3], Angel Galabov[2]

[1] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
[2] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
[3] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences
[4] Institute of Pharmacy, University of Innsbruck, Austria

Human enteroviruses, distributed worldwide, are causative agents of a broad spectrum of diseases with high morbidity, including a series of severe illnesses that affect the CNS, heart, skeletal muscles, and so on. There is no specific treatment available for these infections, and the patients' treatment is mainly supportive. Our team has developed an experimental treatment strategy based on consecutive alternating application (CAA) of enteroviral inhibitors. This work represents the antiviral activity of triple combinations of anti-enteroviral compounds applied via CAA course against Coxsackievirus B3 (Woodruff strain) (CV-B3) infection in newborn mice. Antiviral combination effects were examined by relying on triple CAA combinations consisting of pleconaril (Pl), pocapavir (Po) and vapendavir (V) (Pl/Po/V) or pleconaril (Pl), MDL-860 (M) and oxoglaucine (O) (Pl/M/O) against CV-B3 infection in ICR newborn mice infected s.c. with 20 MLD_{50}. Cumulative mortality (percentage), mean survival time (MST) (days) and weight (in grams) of suckling mice were recorded.
The results of these analyses indicate improved efficacy of Pl/M/O combination administered according to the CAA treatment schedule in CV-B3 infected mice – decreased mortality rate and extended mean survival time (MST). Pl/Po/V applied consecutively were ineffective. In comparison with placebo groups the monotherapeutic course with pleconaril demonstrated some independent antiviral effect. It was found that pocapavir, vapendavir, MDL-860 and oxoglaucine monotherapies were without a protective effect.

P069 *In vitro* evaluation of double combinations against Coxsackievirus B3 and Poliovirus

Adelina Stoyanova[1*], Simeon Galabov[1], Stefan Philipov[2], Angel Galabov[1]

[1] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
[2] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences

The human enteroviruses (EV) comprise one group of the picornavirus family. These EVs cause a diverse array of clinical features, including aseptic meningitis; hand, foot, and mouth disease; neonatal sepsis-like disease; pancreatitis; encephalitis; myocarditis and pericarditis; paralysis and respiratory diseases. The best known members are the polioviruses (PV) and coxsackieviruses. There currently are no EV-specific drugs available for clinical use. One of the reasons is

Evaluation of conventional methods versus whole-genome sequencing and MYCOTBI for the detection of resistance to rifampicin and isoniazid in *Mycobacterium tuberculosis* complex strains from Lithuania and Poland

P106

Laima Vasiliauskaitė[1]*, Zofia Bakuła[2], Edita Vasiliauskienė[1], Daiva Bakonytė[3], Przemysław Decewicz[4], Mikołaj Dziurzyński[5], Małgorzata Proboszcz[6], Edita Valerija Davidavičienė[7], Birutė Nakčerienė[7], Rafał Krenke[6], Tomas Kačergius[1], Petras Stakėnas[3], Tomasz Jagielski[4]

[1] Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Vilnius University; Lithuania
[2] Institute of Biotechnology, Life Sciences Center, Vilnius University; Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland
[3] Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[4] Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland
[5] Department of Biology (DBIO), University of Florence, Italy
[6] Department of Internal Medicine, Pulmonology, and Allergology, Warsaw Medical University, Poland
[7] Department of Programs and State Tuberculosis Information System, Vilnius University Hospital Santaros Klinikos, Lithuania

Rapid and accurate rifampicin (RIF) and isoniazid (INH) resistance detection is essential for the treatment of multi-drug-resistant tuberculosis (MDR-TB). The objective of this study was to evaluate the performance of phenotype- and genotype-based drug susceptibility testing (pDST/gDST) methods for detection of RIF/INH resistance in relation to whole genome sequencing (WGS).

The study included 185 *Mycobacterium tuberculosis* isolates (124 MDR-TB; 61 drug-susceptible), recovered from patients in Lithuania (n = 122) and Poland (n = 63) (2018–2021). DST was performed using BACTEC MGIT-960 System and Löwenstein-Jensen (LJ) medium and MICs were determined using MYCOTBI plates. Line probe assay (LPA) was used for rapid resistance prediction. WGS was done with Illumina NovaSeq 6000 sequencer, and data were analyzed using either (I) bioinformatic pipeline or (II) freely available software platforms (Mykrobe, TB Profiler). The congruency of BACTEC/LJ with LPA was higher than with pipeline-based WGS results (98.9% [RIF]; 97.8% [INH] vs. 97.3% [RIF]; 97.3% [INH]). MIC ranges for genotypically susceptible isolates were ≤0.12–0.5 µg/ml and ≤0.03–0.06 µg/ml for RIF and INH, respectively. The most prevalent *rpoB* mutation was S450L (79.8%), and the majority of mutants demonstrated MICs of >16 µg/ml. *katG* S315T (85.3%) mutants and those with an extra *inhA* C-15T (12.1%) or *inhA* T-8A (0.8%) mutation had MICs within the range of 0.12–>4.0 µg/ml. Single *inhA* C-15T mutant had an MIC of 1 µg/ml.

The overall discordance rate between pDST and gDST results was 3.8%. Five isolates were found that displayed unexplained discordant results. This may relate to heteroresistance, mixed infection or methodological flaws.

BEST ABSTRACT SESSION

PATHOGENS

Phagosomal acidification and autophagy are required to kill Streptococcus pneumoniae in a zebrafish model

Tomasz Prajsnar, Bartosz Michno, Niedharsan Pooranachandran, Andrew Fenton, Tim Mitchell, David Dockrell, Stephen Renshaw

ANIMALS

Zoonotic Acinetobacter baumannii: exploratory epidemiological investigation towards "One Health" approach

Saranya Adukkadukkam, Jayaseelan Murugaiyan

SYSTEMS

Genome wide dissection of secondary chromosome initiation network in Vibrio cholerae

Théophile Niault, Didier Mazel, Marie-Eve Val

THERAPY

Investigation of the protective effect of monoclonal antibodies against poly-N-acetyl glucosamine (PNAG), a superficial polysaccharide, as an alternative to antibiotic-containing eye drops for the prevention of neonatal eye infections caused by Neisseria gonorrhoeae

Cagla Bozkurt Guzel, Ozlem Oyardi, Tanweer Zaidi, Gerald Pier

ENGINEERING

Efficiency of bio-hydrogen production as a result of dynamics of metabolic activity and composition of dark fermentation microbial communities: open-flow systems vs static batch experiments

Anna Detman, Daniel Laubitz, Aleksandra Chojnacka, Anna Sikora

UBIQUITY

PICOTA: an extendible pipeline for identification of composite transposons from assembly graphs

Recep Can Altinbag, Ulas Tezel

RESISTANCE

The effect of rotating magnetic field on susceptibility of methicillin resistant Staphylococcus aureus strains exposed to activity of antibiotics

Karol Fijałkowski, Marta Woroszyło, Daria Ciecholewska-Juśko, Adam Junka