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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

EXTENDEND ABSTRACT OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Antimicrobial phthalocyanine activated by indoor light

Alexander Efimov[1]*, Zafar Ahmed[1], Lijo George[1], Ville Santala[1], Natalya Grammatikova[2]

[1] Tampere University, Faculty of Engineering and Natural Sciences, Korkeakoulunkatu 10 33720, Tampere Finland; [2] Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, Bolshaya Pirogovskaya Str 11, Moscow-119021, Russia

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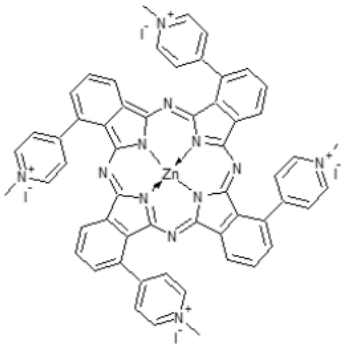
ABSTRACT

Photosensitizers, such as porphyrinoids and phenothiazines, can be efficient in photodynamic treatment of drug-resistant bacteria, fungi and biofilms. However, it usually requires the use of special light sources of high intensity such as lasers, and commonly suffers from fast bleaching. We report the Zn complex of novel tetracationic phthalocyanine with four pyridyl substituents, which is activated by an inexpensive light-emitting diode lamp or by consumer-grade fluorescent lamps. Antimicrobial efficacies are extremely high, allowing to inactivate up to 99.9999% of initial populations of drug-resistant *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baylyi*, *Enterococcus faecalis*, and *Candida albicans*. Inactivation occurs on the surface of dyeimpregnated cellulose support with the load of chromophore as small as 0.1 g/m^2. The required illumination time is 15-60 minutes. The immobilized dye is well resistant to leaching and bleaching.



ZnPc

Exp. conditions	*E.Faecalis 583*		*MRSA 88*	
	30 min	**60 min**	**30 min**	**60 min**
Room light 270 lux	99%	99.999%	99%	99.999%
LED light 4000 lux	99.99%	99.9999%	100%	100%

Acknowledgments:

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References:

L. George, A. Mueller, B. Roeder, V. Santala, A. Efimov, Dyes Pigments, 147 (2017) 334-342.
L. George, A. Hiltunen, V. Santala, A. Efimov, J. Inorg. Chem. 183 (2018) 94-100.

Correspondence: Email - alexander.efimov@tuni.fi

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Antibacterial and antibiofilm activities of the NSAID acetylsalicylic acid against *Escherichia coli* and *Staphylococcus aureus*

Cláudia Leão[1], Anabela Borges[1]*, Diana Oliveira[1,2], Manuel Simões[1]

[1] LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr Roberto Frias, s/n, 4200–465 Porto, Portugal; [2] CIQUP - Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

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ABSTRACT

Multi-drug resistance has been growing abruptly worldwide. Moreover, the development of new antibiotics is scarce and finding new antibacterial drugs is becoming increasingly difficult. In fact, this process can be risky as requires high investments by pharmaceutical industries. Therefore, new approaches to counteract the global threat of bacterial resistance, including in the sessile mode of growth (biofilms), are needed [1]. Repurposing old drugs for new treatment purposes can be an excellent alternative with lesser potential clinical implications [2]. In this study, the antimicrobial and antibiofilm activities of a non-steroidal anti-inflammatory drug (NSAID), acetylsalicylic acid, against *Escherichia coli* and *Staphylococcus aureus* was evaluated. The minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined by the broth microdilution method and culturability on plate count agar, respectively. Its potential to eradicate pre-formed *E. coli* and *S. aureus* biofilms (24-h old) was performed using a microtiter plate assay and characterized in terms of biofilm mass (crystal violet staining)/metabolic activity (alamar blue staining) reductions and culturability. The MIC values were 1750 μg/mL and 2000 μg/mL for *E. coli* and *S. aureus*, respectively. The MBC was found to be > 2000 μg/mL (the maximum concentration tested) for both bacteria. No biofilm mass removal was observed. However, acetyl salicylic acid promoted metabolic activity reductions higher than 70% for all concentrations tested (MIC, 5 × MIC and 10 × MIC). In terms of culturability, a dose dependent effect was obtained, with 3.6-log CFU (colony-forming units) per cm^2 reduction at MIC and total loss of culturability at 5 × MIC and 10 × MIC for both bacteria. Overall, the results obtained suggested that non-antibiotic drugs such as acetyl salicylic acid might be an interesting alternative and/or complement for antiinfective therapeutic approaches for a post-antibiotic era.

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References:

[1] A. Borges, A. Abreu, C. Dias, M. Saavedra, F. Borges, M. Simões, Molecules 21 (2016) 877.
[2] I. M. Oliveira, A. Borges, F. Borges, M. Simões, European Journal of Medicinal Chemistry166 (2019) 197-205.

Correspondence: Email - apborges@fe.up.pt

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Nontuberculous mycobacterial musculoskeletal infections: a caseseries from a tertiary referral center

Noah Goldstein[1], Ben St. Clair[1], Shannon H. Kasperbauer[1,2], Charles L. Daley[1,2], Bennie Lindeque[1]*

[1] University of Colorado, Aurora, Colorado, USA; [2] National Jewish Health, Denver, Colorado, USA

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ABSTRACT

Nontuberculous mycobacteria represent an uncommon but important cause of infection of the musculoskeletal system. Such infections require aggressive medical and surgical treatment and are often complicated by delayed recognition and diagnosis. We retrospectively reviewed all 14 cases of nontuberculous mycobacterial musculoskeletal infections treated over a 6-year period by orthopedic surgeons at a university-affiliated tertiary referral center. All patients required multiple anti-microbial agents as well as aggressive surgical treatment, with 13 of 14 patients ultimately achieving cure. Four patients required amputation for adequate control of infection. Half of our patients were immunosuppressed at presentation, either by medications or other medical illness. Six infections involved joint prostheses, and all ultimately required hardware explantation and placement of an antibiotic spacer for eradication of infection. Our series highlights the importance of vigilance for nontuberculous mycobacterial musculoskeletal infection, particularly in patients who are immunosuppressed or have a history of musculoskeletal surgery.

References:

D. R. Prevots, P. A. Shaw, D. Strickland, L. A. Jackson, M. A. Raebel, M. A. Blosky et al. Am J Respir Crit Care Med. 182 (2010) 970–6.
J. Adjemian, K. N. Olivier, A. E. Seitz, S. M. Holland, D. R. Prevots. Am J Respir Crit Care Med. 185 (2012) 881–6.
M. Al-Houqani, F. Jamieson, M. Mehta, P. Chedore, K. May, T. K. Marras. Chest. 141 (2012) 190–7.
A. B. Spaulding, Y. L. Lai, A. M. Zelazny, K. N. Olivier, S. S. Kadri, D. R. Prevots, et al. Ann Am Thorac Soc. 14 (2017) 1655–61.
D. E. Griffith, T. Aksamit, B. A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, et al. Am J Respir Crit Care Med. 175 (2007) 67–416.
G. S. Smith, A. J. Ghio, J. E. Stout, K. P. Messier, E. E. Hudgens, M. S. Murphy, et al. J Infect. 72 (2016) 678–86.
P. M. Cassidy, K. Hedberg, A. Saulson, E. McNelly, K. L. Winthrop. Clin Infect Dis Off Publ InfectDis Soc Am. 49 (2009) 124-129.

Correspondence: Email - bennie.lindeque@ucdenver.edu

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Sequencing Applied to Modern Clinical Microbiology

C. Sabença[1,2*], R. Beyrouthy[3,4], B. Richard[3,4], P. Poeta[5,6], G. Igrejas[1,2,5]

[1] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Unit of Functional Genomics and Proteomics, University of Trás-os-Montes and AltoDouro, Vila Real, Portugal; [3] National Reference Center for Antibiotic Resistance, Clermont-Ferrand, France; [4] University Hospital Center, Clermont-Ferrand, France; [5] LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Caparica, Portugal; [6] Department of Veterinary Science, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

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ABSTRACT

Multi-Antimicrobial resistance is a global and multifaceted public health issue, which requires a multidisciplinary and holistic approach as the pandemic spread and evolution of highly resistant bacteria occurs similarly in the human, animal and environmental settings. *Escherichia coli* that produces extended-spectrum β-lactamases (ESBL) are one of the major public health concerns [1]. ESBL, along with other resistance genes, are located on plasmids giving them the ability to disseminate those resistance genes to other bacterial species [2], including in isolates from wild animal populations [3]. Even though wild animals are not in direct contact with antibiotics, they are infected by the excessive use in humans and veterinary medicine[4]. A total of 39 Enterobacteriaceae strains were selected from our collection of bacterial isolates from different wild and domestic animals previously studied. The strains were identified using mass spectrometry. The susceptibility test was performed on 30 antibiotics and ESBL production was detected by both the combination disc test and the double-disc synergy test according to EUCAST standards. We determined the whole-genome sequences of strains by using de novo assembly of 2 × 150-bp paired-end reads generated by using sequencing technology by Illumina. Of the 39 strains, 22 were ESBL-producing E. coli. All strains presented multiresistance and the most frequent ESBL mechanism was the CTX-M-1 and it was associated to IncI1 plasmid. Therefore, ESBL-producing E. coli has disseminated in several species, including in birds that can be considered spreaders of antibiotic resistance since they can migrate long distances in short periods of time posing a serious risk for the global spread of multidrug-resistant bacteria.

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References:

[1] A.A. Shah, F. Hasan, S. Ahmed, A. Hameed, Research in Microbiology (2004).
[2] H. Radhouani, N. Silva, P. Poeta, C. Torres, S. Correia, G. Igrejas, Frontiers in Microbiology (2014).
[3] A. Garcês, S. Correia, F. Amorim, J.E. Pereira, G. Igrejas, P. Poeta, Journal of Hazardous Materials (2017).
[4] M. Dolejska, C.C. Papagiannitsis, Plasmid (2018).

Correspondence: Email - carolinasabenca@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Taking Antibiotics: A Model of How It Works

Eva M. Ogens

Ramapo College of New Jersey

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ABSTRACT

Almost all children have taken antibiotics as a result of ear infections, strep throat, or other bacterial infections. Some of them feel better soon and don't understand why they have to keep taking the medication for the full ten days as prescribed. Others forget to take the medicine, and then often have to be put on a stronger type of antibiotics. This game enables students to experience a model of the effects of antibiotics on a population of disease-causing bacteria during an infection. Students learn how variables such as skipping a day of medication affect the persistence of the disease. A key concept is that almost every naturally occurring population of bacteria that cause disease has a component that is resistant to antibiotics. By graphing data, students can visually understand why it is important to take a complete course of antibiotics to kill all the bacteria and decease the likelihood of bacteria becoming resistant, which can be harmful to human health and is a major public health problem.

References:

Eva M. Ogens, Richard Langheim. The American Biology Teacher. 78 (2016) 568-574.

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Correspondence: Email - eogens@ramapo.edu

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Opinions and regularity conclusions on drug combination to prevent resistance

Ganjun Yuan*, Pingyi Li, Houyi Qin, Xuejie Xu, Xiaoyuan Song, Sheng Cao, Su He, Shan Lai

College of Bioscience and Bioengineering, Jiangxi Agricultural University, Nanchang 330045, China

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ABSTRACT

Multi-Antimicrobial resistance seriously threatened human health and economic development. Combination therapy is generally proved to be an effective strategy to fight resistance, while no regularity conclusion could be drawn to guide its practice, and even some data on its effects are conflicting. To further explore it, the fractional inhibitory concentration indexes (FICIs) of three combinations against methicillin-resistant *Staphylococcus aureus* (MRSA) were determined using checkerboard method, and their minimal concentrations inhibiting colony formation by 99% ($MIC_{99\%}$s) and mutant prevention concentrations (MPCs) alone or in combinations including different proportions were determined using agar plates. The results led to the discovery of regularity conclusions in drug combinations to prevent resistance [1]: (1) The MSW of one agent is closely related to the proportion of two agents, and different proportions of a combination would present different MPCs and mutant selection window (MSWs). Thereby, the MSWs of one antimicrobial agent can be narrowed by combining with another whether it is synergistic or not. This can explain various results of drug combinations to prevent or fight resistance at present, and even contrary ones [2,3]. Mainly depended on the proportion of two agents, many combinations had enough potential to prevent resistance [2-4], and even that the susceptibility of one antimicrobial agent might be enhanced by another in an antagonistic combination [5], while some combinations may result in high mutational frequencies, such as levofloxacin in combination with lower dose of colistin [4]. (2) The smaller the FICIs of two agents in combinations were, the more probable their MSWs were to close each other, and the greater the potency to prevent or delay resistance according to MSW and MPC hypotheses [6]. Thus, discovering remarkably synergistic combinations closing each other's MSWs were our goals. As two antimicrobial agents in a certain combination usually presented different pharmacokinetics parameters in vivo, their proportions in blood and infectious tissue would accordingly change, and thus lead to their different MPC and MSWs. This must fluctuate or even invert the practical effects preventing resistance, and increased the complexity and uncertainty of drug combination preventing resistance. However, some opinions and measurements can be referred in the practice preventing antimicrobial resistance based on above regularity conclusions. (1) We might select two agents with similar pharmacokinetics parameters as possible as we could for synergistic combination to prevent resistance. (2) As remarkably synergistic combinations would be more favorable to prevent resistance, a new antimicrobial agent synergistically combining with one or more, as a regular combination like the application of anti-tuberculosis drugs, should be encouraged to be approved, and even as a hybrid antibiotic such as rifamycin-quinolone. (3) Antimicrobial agents targeting identical macromolecular biosynthesis pathway with different sites had a great potency to discover synergistic combinations, such as roxithromycin/doxycycline (respectively targets ribosomal protein 50S and 30S subunits), and trimethoprim/sulfamethoxazole (respectively targets dihydrofolate synthase and reductase) used for a long time. (4) We can select a weak one to narrow the MSWs of a remarkable one to prevent resistance by greatly increasing the proportion of weak one in a combination whatever synergistic one or not, while synergistic one is better. For example, one or more natural products from plants, herbs and traditional chinese medicines can be considered.

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References:

[1] X. Xu, L. Xu, G. Yuan, Y. Wang, Y. Qu, M. Zhou, Sci Rep 8 (2018) 7237.
[2] M.A. Fischbach, Curr Opin Microbiol 14 (2011) 519–523.
[3] M. Vestergaard, W. Paulander, R.L. Marvig, J. Clasen, N. Jochumsen, S. Molin, L. Jelsbak, H. Ingmer, A. Folkesson. Int J Antimicrob Age. 47 (2016) 48–55.
[4] G.G. Zhanel, M. Mayer, N. Laing, H.J. Adam, Antimicrob Agents Chemother 50 (2006) 2228–2230.
[5] M. Baym, L.K. Stone, R. Kishony, Science 351 (2016) aad3292.
[6] K. Drlica, X. Zhao, Clin Infect Dis 44 (2007) 681–688.

Correspondence: Email - gyuan@jxau.edu.cn

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Strategies to improve infection control and to limit antibiotic resistant infectious agents in dentistry

Livia Barenghi[1], Alberto Barenghi[1,2], Alberto Di Blasio[2]

[1] Integrated Orthodontic Services S.r.l., Via Camillo Benso di Cavour 52 C, 23900 Lecco, Italy; [2] Department of Medicine and Surgery, Centro di Odontoiatria, Parma University, ViaGramsci 14, 43126 Parma, Italy

Available Online: 15 November 2019

ABSTRACT

Nowadays, other than blood-borne viruses and water-borne bacteria, antibiotic-resistant bacteria are a significant hazard in dentistry when taking into account the worldwide overuse of antibiotics, the limited awareness on infection prevention guidelines, and the very frequent lapses and errors during infection prevention. Data sustains the evidence of possible reservoirs of antibiotic resistant bacterial infections in humans (dental staff and patients) and on dental items in dental offices. We take into account Staphylococci and Enterobacteriaceae as markers since they are considered prioritized bacteria according to antibiotic resistance pressure, and are able to adapt to different closed habitat environments (from mechanically ventilated rooms in health-care facilities to very extreme ones (such as spaceflight)). Furthermore, there is available data for dental settings and on their virulence factors. In particular, MRSA plays a key role in its colonization in patients and dental workers, presence on gloves, resistance (days-months on dry inanimate surfaces), the contamination of different clinical contact surfaces in dental settings, the ability of some strains to produce biofilm and finally, its low estimated infective dose. Moreover, an alarming genetic similarity has been shown between MRSAs isolated in dental clinics and some EMRSA clones (EMRSA-15 and EMRSA-16 lineage). For better healthcare personnel and dental patient safety, we need: 1) to improve knowledge on bioburden and biofouling, also based on molecular biological methods; 2) education and training initiatives; 3) implementation of infection control prevention according to guidelines; 4) to limit the hazards in surgical dental settings and HA-MRSA infections.

References:
[1] L. Barenghi, A. Barenghi, A. Di Blasio, InTechOpen (2018).
[2] L. Barenghi, A. Barenghi, A. Di Blasio, InTechOpen (2018).
[3] C. Urbaniak, A. Checinska Sielaff, K. G. Frey, et al. Scientific Reports 8 (2018) 1-13.

Correspondence: Email - livia.barenghi@libero.it

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Gold nanoparticles as alternative therapy for antibiotic-resistant bacterial strains

María Cecilia Becerra[1,2*], María Jazmín Silvero[1,2], Diamela Rocca[2], Virginia Aiassa[2,3], Juan C. Scaiano[4]

[1] IMBIV-CONICET; [2] Dpto. de Cs. Farmacéuticas. FCQ, Universidad Nacional de Córdoba. Haya de la Torre S/N, Córdoba, Argentina; [3] UNITEFA-CONICET; [4] Departmentof Chemistry and Biomolecular Sciences and CAMaR, UO, Canada.

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ABSTRACT

A strategy to combat infections by bacteria resistant to antibiotics is the development of nanomaterials with photosensitizing activity. In our group, gold nanoparticles (NPS) stabilized with amoxicillin (amoxi@AuNPs) were obtained[1]. They have the advantage of being synthesized in a single step and in a few minutes. The evaluation of the antibacterial activity against methicillinsensitive *Staphylococcus aureus* (MSSA ATCC 29213) and a methicillin-resistant clinical strain (MRSA 9455) was carried out by irradiation with white light LEDs (to cover the absorption of the different shapes of NPs) and subsequent counting in solid medium of the colony forming units. The eradication of biofilms from clinical strains of MRSA and *Pseudomonas aeruginosa* (PAE) treated with amoxi@AuNPs was quantified. The biomass of the biofilm was quantified in the clinical strains MRSA 771 and 773 and in the clinical strains PAE 191150 and 189718, by the staining test with crystal violet. Metabolic activity was determined by reducing the XTT reagent and SEM images. To investigate the mechanism of action of the amoxi@AuNPs, the generation of reactive oxygen species (ROS) in *S. aureus* was measured using the dihydrorhodamine 123 (DHR) probe. Results suggest that maximum antibacterial effect was achieved at 30 min of irradiation, with a concentration of 1.5 µg/mL amoxi@AuNPs. A marked reduction in the metabolic activity of the biofilms treated with amoxi@AuNPs and irradiated was obtained. The metabolic activity was reduced with respect to its untreated controls. The results were corroborated by SEM images. Fluorescence microscopy with temporal resolution evaluated the activity of the mentioned NPs in co-cultures of bacteria and eukaryotic cells. These NPs possess bactericidal activity and an excellent biocompatibility in co-cultures. In order to give a step towards the application of this technology, we have synthesized in just one step a gel containing AuNPs plus an antimicrobial peptide (AMP). Casein was the chosen AMP because it is a small biocompatible molecule, relatively cheap and with gelation properties. SEM images showed spherical NPs (10 ± 2 nm diameter) and were stabilized between the casein net. They have an absorption peak at 544 nm and inhibit the growth of pathogenic strains as *Klebsiella pneumoniae* and *S. aureus* after only 15 min with green LEDs. This is possible because of the combination effect of the AMP and the plasmon excitation. The application of these nanoparticles for Photodynamic Antimicrobial Therapy is promising to treat infections resistant to antibiotics given its high stability *in vivo*, cytocompatibility and also because, until now, the development of resistance has not been registered.

Acknowledgments:

CONICET, SECyT-UNC, FONCYT.

References:

[1] M.J.Silvero, et al. ACS Omega 3 (2018) 1220−1230.

Correspondence: Email - becerra@fcq.unc.edu.ar

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

One Health Approach for Identifications of Sources/Reservoirs of Multidrug Resistant Potential Pathogens in Wild Animals and their Environment

Marilyn C. Roberts[1], Daira Melendez[1], Alexander L. Greninger[1], Samuel Wasser[1], Randall C. Kyes[1], Stefan Monecke[2], Ralf Ehricht[2], Prabhu Raj Joshi[3]

[1] University of Washington, Seattle WA USA; [2] Leibniz Institute for Photonic Technologies, Jena Germany; [3] Nepalese Farming Institute, Kathmandu Nepal

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ABSTRACT

Bacteria such as extra-intestinal pathogenic *E. coli* (ExPEC) and methicillin-resistant *S. aureus* (MRSA) are important opportunistic pathogens. They might belong to pandemic, epidemic and/or sporadic clones. Some f the clones are associated with humans, others are associated with wild and/or domestic animals. Some clones are shared by both and may be found contaminating the environment. In this study, we examined the spread of ExPEC and MRSA isolates in a One Health Approach to better understand the sources/reservoirs and possible transmissions of potential pathogens. *E. coli* was isolated from fresh fecal samples collected from the endangered Southern Resident Killer Whales (SRKW) population (*Orcinus orca*) in 2013. Nine distinct isolates were recovered from 7 SRKW individuals with whole genome sequencing, de novo assembly and analysis done. Eight were multidrug resistant ExPEC ST73 clonotype C24:H10 isolates taken from 7 individuals from 3 pods. The ninth isolate was not antibiotic resistant and was ExPEC ST127 clonotype C12:H2. All carried a variety of virulence genes which differed between the ST73 isolates and between the ST73 and the ST127 isolate. Previous studies have shown that the Puget Sound (Salish Sea), the home to the SRKW, is contaminated with multiple ARGs and antibiotic residues, especially near waste water treatment plant discharge sites. Their food source, Chinook salmon carry antibiotic residues in their tissue. In 2018, MRSA was non-invasively collected from macaque (*Macaca mulatta*) saliva samples (n=13) and environmental samples (n=19) near temple areas in Kathmandu. MRSA (n=5) from human wound infections in a Kathmandu hospital were also collected. All 37 isolates were characterized using The Alere StaphyType® DNA microarrays1. Twenty-three (62%) were MRSA CC22 SCCmec type IVa previously found in Nepalese macaque of human origin and insolated from monkey (n=4; 31%), environmental (n=14; 74%), and human (n=5; 100%) samples. Eight monkey MRSA were CC361 SCC*mec* type IVa. One MRSA isolated from a monkey and environment were CC88 SCC*mec* type V, previously found in Nepalese swine samples2. The remaining environmental MRSA included one each, CC121 SCC*mec* type V, and CC772 SCC*mec* type V, all of human origin and 2 CC779 SCC*mec* type V, potentially a novel clone. All 37 MRSA carried the bla gene, 31 carried the *aacA-aadD*, 25 *dfrA* and 21 *erm*(C) genes. All CC22 isolates carried the *aacA-aadD*, *dfrA* and 17 carried the *erm*(C) genes, while 2 MRSA from macaque, 3 MRSA from environmental and 1 human MRSA lacked the *erm*(C) gene. The 1 macaque and environmental CC88 MRSA both carried the *aacA-aphD* gene but only the macaque MRSA carried the *aphA3* and sat resistance genes, neither previously identified in primate MRSA. Among the 23 CC22 MRSA, 21 carried the PVL locus and *tst* virulence gene which is unusual and include all the monkey and human isolates and 12 of 14 environmental isolates. This current study suggests that humans are the source of the MRSA identified both in the macaques and the environment and may be linked to humans feeding the primates. The most likely source of the ExPEC isolates in the SRKW is either directly acquired from pollution in the Salish Sea, or from their salmon diet. It is unknown if the ExPEC cause disease in the SRKW or if they contribute to the ongoing decline of this endangered species.

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References:

[1] S. Monecke, L. Jatzwauk, E. Müller, H Nitschke, K Pfohl, P. Slickers, et al. PLoS One 11 (2016).
[2] M.C. Roberts, P.R. Joshi, A.L. Greninger, D. Melendez, S. Paudel, M. Acharya, et al. FEMS Microbiol Eco.(2018).

Correspondence: Email - marilynr@uw.edu

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Associations between infection and colonization and opportunistic antibiotic-resistant *Klebsiella pneumoniae*

Patricia Severino[1*], Leticia Busato Migliorini[1], Aline Silva Muniz[1], Fernando Gatti de Menezes[2], Marinês Dalla Valle Marino[3]

[1] Albert Einstein Research and Education Institute, Hospital Israelita Albert Einstein, Sao Paulo, Brazil ; [2] Infection Control Service, Hospital Israelita Albert Einstein, Sao Paulo, Brazil; [3] Microbiology Laboratory, Hospital Israelita Albert Einstein, Sao Paulo, Brazil

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ABSTRACT

Multidrug resistant bacteria (MDR) are difficult to eradicate and spread rapidly. In Brazil, Carbapenem Resistant Enterobacteria (CRE), mainly those producing *Klebsiella pneumoniae carbapenemase* (KPC), are considered epidemic [1]. Efficient antibiotic therapies against MDR tend to become scarce, so studies aiming to understand patterns of dissemination of CRE, and specifically of CRE KPC, are necessary [2,3]. In this work we correlate gastrointestinal and lower respiratory tract colonization with CRE and CRE KPC with hospital acquired infections. We analyzed records of CRE and CRE KPC detected by routine surveillance carried out the Infection Control Program as well as of CRE and CRE KPC isolated from samples taken for culture from infected patients (blood, bronchoalveolar lavage, tracheal aspirate and urine). Cases were defined as patients clinically infected or colonized with KPCproducing *K. pneumoniae* (KPC-KP) and Carbapenem Resistant *K. pneumoniae* (CR-KP) admitted in a large nonteaching hospital between January 2007 and December 2017. A time-series analysis based on autoregressive integrated moving average (ARIMA) was used to identify trends in antibiotic resistance incidence. The detection of CR-KP preceded the detection of KPC-KP in this period, but similar patterns of incidence were observed after 2011. We identified an increasing trend in resistance to Carbapenems ($p < 0.01$) and in KPC-KP ($p < 0.003$) and MDR KP ($p < 0.02$) detection. CR-KPand KPC-KP-colonized patients were tracked for infection and results suggest rising prevalence of CR-KP and KPC-KP, and asymptomatic carriage as important risk factors for infection following colonization. However, these factors alone do not explain the observed scenario. Whole genome sequencing will be used to further characterize endemic, epidemic and MDR clones. Gastrointestinal colonization has long been recognized as a reservoir for strains causing hospital acquired infections [4], but only recently we have the tools, by means of whole genome sequencing, to further characterize *K. pneumoniae* and expand our understanding on differences in infection risk and outcomes regarding specific clones.

Acknowledgments:

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References:

[1] M. G. Quiles et al. Brazilian Journal of Medical and Biological Research. 48 (2015)174–177.
[2] T. Ducomble et al. Journal of Hospital Infection 89 (2015)179–185.
[3] T. Kaiser et al. Am J Infect Control. 46 (2018) 54-59.
[4] R. Selden, S. Lee, W. L. Wang, J. V. Bennett, T. C. Eickhoff. Ann. Intern. Med. 74 (1971)657–664.

Correspondence: Email - patricia.severino@einstein.br

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Upshots of a decennium of antimicrobial resistance proteomics

Susana Correia[1-4]*, Patrícia Poeta[3,4], Luís Pinto[1-4], Gilberto Igrejas[1-3]

[1] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Portugal;[4] Veterinary Science Department, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

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ABSTRACT

Antimicrobial resistance (AMR) is acknowledged today as one of the most concerning threats to global human health and the world has now started to act concertedly to tackle this problem. However, AMR is not a recent problem being in fact as old as the discovery and use of antibiotics. For the last 10 years, the Functional Genomics and Proteomics Unit based at the University of Trás-os-Montes and Alto Douro, Vila Real, Portugal, has been aiming to better understand the mechanisms by which bacteria survive antibiotic action by looking into the entire complement of proteins expressed by resistant strains. Using different perspectives, the proteomics studies performed have contributed with the knowledge associated with over 2000 proteins that can, singlehandedly or as a complex whole, hold the key for new insights to unravel AMR. Since 2009, the proteomes of different *Salmonella, Enterococcus, Escherichia coli and Staphylococcus aureus* strains have been thoroughly studied with the purpose to identify either the main proteins present or those differentially expressed between strains. By looking at the whole proteome or subfractions of the proteome, studies have been performed to compare resistant strains with different levels of resistance, with related non-resistant strains and in the presence and absence of antibiotic stress. Protein separation with high resolving power has been achieved with 2D gel electrophoresis (2-DE) and shotgun analysis has allowed to overcome solubility limitations of membrane proteins. Hence, a comprehensive coverage of the present proteins has been attained by using 2-DE followed by either matrix-assisted laser desorption/ionization-time of flight (MALDITOF)- or liquid chromatography (LC)- tandem mass spectrometry (MSMS) together with shotgun LC-MSMS approaches. The study of strains recovered from clinical human samples provided a better understanding of extended-spectrum beta-lactamase (ESBL) producing *E. coli*, new insights into pleural empyema methicillin-resistant *S. aureus* (MRSA) and an in-depth examination of the mechanisms of quinolone resistance in *Salmonella Typhimurium* causing acute gastroenteritis. Multidrug resistant (MDR) *E. coli*, ciprofloxacin resistance and vancomycin resistant enterococci were further unravelled through the proteomes of samples recovered from pigs slaughtered for human consumption. Also, the role of resistant bacteria as environmental reservoirs of AMR was better elucidated though the proteomes of ESBL-positive *E. coli*, vanA-positive enterococci and MDR *Salmonella* recovered from a variety of free-ranging wild animals including boars, rabbits, seagulls, red foxes and Iberian wolf and lynx. Proteomic approaches have considerably improved during the past decade, being successfully used to investigate protein expression profiles. By greatly contributing to a better understanding of the specific mechanisms that contribute to AMR, proteomics has proved to be the appropriate research tool to overcome this major modern medicine challenge.

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Correspondence: Email - scorreia@utad.pt

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Maldi-Tof mass spectrometry applied to modern clinical microbiology: research and identification of biomarkers in multiresistant bacterial species

Telma de Sousa[1-4*], Didier Viala[5], Laëtitia Théron[6], Christophe Chambon[5], Michel Hébraud[5,7], Patrícia Poeta[2,3], Gilberto Igrejas[1,3,4]

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Animal and Veterinary Science, University of Trás-os-Montes and AltoDouro (UTAD), Vila Real, Portugal; [3] LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Caparica, Portugal; [4] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [5] INRA, Plateforme d'Exploration du Métabolisme (PFEM), France; [6] INRA, UR QuaPA, F-63122 Saint-Genès Champanelle, France F-63122; [7] Université Clermont Auvergne, INRA, UMR MEDiS, France.

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ABSTRACT

Antimicrobial resistance is a global and multifaceted public health issue, which requires a multidisciplinary and holistic approach as the pandemic spread and evolution of highly resistant bacteria occurs similarly in the human, animal and environmental settings. *Escherichia coli* and *Enterococcus* spp., as commensal bacteria, are usually not responsible for diseases, but with the acquisition of resistance genes to various antibiotics they can be serious. These bacteria also have a great capacity to spread these same genes, sometimes to phylogenetically distant bacteria, which represents a serious public health problem inthe world. Among the pathogens are *Escherichia coli* Extended-Spectrum β-Lactamase (ESBL) and Vancomycin-Resistant Enterococci (VRE). This work aims to use the analytical potential of MALDI-TOF mass Spectrometry (MS) to characterize *Escherichia coli* and *Enterococcus* spp. isolates and identify protein biomarkers associated with antibiotic resistance. This would allow rapid and cost-effective identification of resistance carried out by pathogenic strains in order to more effectively treat patients and/or better understand the spread of these resistances. The 33 samples of*E. coli* (ESBL) are from various animals and 22 *Enterococcus* (VRE) are samples of various types of processed meat. All the samples showed multiresistance to the various antibiotics and these results are consistent compared to studies carried out on these types of bacteria. In a second step, aMALDI -TOF MS approach was implemented, not only to characterize strains but mainly to identify biomarkers attesting to their resistance to antibiotics. Each strain was grown in the presence or absence of different antibiotics. All strains were prepared according to the Freiwald and Sauer (1) protocol. The protein fingerprints were then determined by MALDI-TOF MS in linear mode over a mass range of 2 to 20 kDa. The resulting data are currently analysed, and the spectra obtained with or without antibiotics are compared by using the ClinProTools™ bioinformatics software. The putative biomarkers of antibiotic resistance will then be evaluated with a wide range of strains and their nature will be determined by other MS approaches.

Acknowledgments:
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References:
[1] A. Freiwald, S. Sauer. Nature protocols (2009) 732-742.

Correspondence: Email - telmaslsousa@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Dissemination of colistin-resistant bacteria with mobile resistance gene *mcr* in a rural community of Vietnam

Yoshimasa Yamamoto[1,2]

[1] Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan; [2] Life Science Research Center, Gifu University, Gifu, Japan

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ABSTRACT

Colistin is typically used as a last-resort treatment when there are no other therapy options available. However, current reports show an increase in colistin-resistant bacteria worldwide due to the abuse of colistin in the livestock sector. Furthermore, the discovery of mobile antibiotic resistance genes, such as *mcr-1*, in 2015 indicates the possibility of further spread of colistin resistance to other bacteria. Therefore, extensive studies on colistin-resistant bacteria possessing *mcr* are being carried out on infectious disease specimens and livestock. The colistin-resistance with mcr represents an emerging global health threat. However, the susceptibility and exposure of local residents living in the areas of frequent usage of colistin in livestock to the colistin-resistant bacteria remains to be studied. The carriage of colistin-resistant bacteria with mobile resistance genes by human residents may increase the risk of acquiring intractable infections. The study was conducted at Nguyen Xa village, Thai Binh province, Vietnam, from November 2017 to February 2018. The village, a representative rural community in Vietnam, had 7,730 residents in 2,008 households in 2015. A total of 98 healthy participants from 36 households were enrolled. One stool specimen was obtained from each participant to test for the presence of colistin-resistant *E. coli*, using a selective medium (CHROMagarTM COL-APSE, CHROMagar, Paris, France). The colistin-resistant bacteria were detected in 70.4% of the residents. All the colistin-resistant isolates were identified as *E. coli*. The proportion of households that had members possessing colistin-resistant *E. coli* was also quite high (80.6%). Sixty-nine of the 70 colistin-resistant *E. coli* isolates possessed either *mcr-1* and/or *mcr-3*. Only one colistin-resistant isolate did not contain any *mcr-1* to *mcr-5* genes. The minimum inhibitory concentrations of *mcr* (+) isolates to colistin were ≥8 μg/ml. Pulsed-field gel electrophoresis analysis indicated no clonal expansion of any specific strain. The majority of *mcr* (+) isolates showed that the rate of multidrug resistance (MDR) of colistin-resistant *E. coli* isolates was 92.8%, which means that they show resistance to at least one antibiotic drug in three or more antibiotic classes. These results revealed the dissemination of MDR colistin-resistant *E. coli*, harboring the colistinresistant mobile gene *mcr* among commensal bacteria of residents, in a rural community in Vietnam. In particular, it is a remarkable finding in the public health viewpoint that most households, which participated in the study, had colistin-resistant *E. coli* carriers. Thus, this requires urgent public health attention.

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Correspondence: Email - yyamamot@sahs.med.osaka-u.ac.jp

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Combating Antibiotic Resistance: Glyconanomaterials, Nanoantibiotics and Drug Repurposing

Mingdi Yan[1]

[1] University of Massachusetts Lowell

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ABSTRACT

The increasing prevalence of drug resistance to the majority of existing antibiotics has generated a pressing global healthcare crisis. Certain highly resistant bacteria have acquired multiple mechanisms against all available antibiotics including the drugs of last resort. We are developing new strategies to combat antimicrobial resistance, including glyconanomaterials, nanoantibiotics and drug repurposing, which will be discussed in this talk. Glyconanomaterials are nanomaterials that present carbohydrates/glycans at their surfaces[1,2]. As such, these materials mimic cell surfaces, which are generally decorated with different glycolipids and glycoproteins that, through recognition of carbohydrate-binding proteins such as lectins and antibodies, mediate a wide variety of cellular communications including bacterial infection. We showed that the interactions of glyconanomaterials with bacteria can be modulated by the carbohydrate on the nanomaterial. For example, trehalose-functionalized glyconanomaterials interact strongly with mycobacteria[3]. The findings have been applied to detect bacteria[4], as well as for the targeted delivery of antibiotics[5]. Pure nanoantibiotics are nanoparticles made entirely of pure antibiotic molecules. Because PNAs are carrier-free, the drug encapsulation efficiency is close to 100%, and the potential burden caused by carrier degradation can be avoided. In a proof-of-concept study, we developed a modular synthesis of ciprofloxacin derivatives and fabricated them into theranostic nanoparticles. These compounds are propeller-shaped, and upon precipitation into water, readily assembled into amorphous nanoaggregates that displayed enhanced luminescence. In addition, the PNAs exhibited up to 2 orders of magnitude enhancement in the antibacterial activity[6]. Finally, our recent work on drug repurposing will be discussed. SAR (structure-activity relationship) studies from in vitro activities against ESKAPE pathogens and mammalian cell cytotoxicity identified lead compounds that show promises for both Gram-negative and Gram-positive strains.

Acknowledgments:

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References:

[1] X. Chen, O. Ramström, M. Yan. Nano Res. 7 (2014) 1381.
[2] O. Ramström, M. Yan. Chem. Eur. J. 21 (2015) 16310.
[3] K. W. Jayawardena, H. S. N. Jayawardena, S. A. Wijesundera, T. De Zoyza, M. Sundhoro, M. Yan. Chem. Commun. 51 (2015) 12028.
[4] X. Chen, B. Wu, K. Jayawadena, N. Hao, H. S. N. Jayawadena, R. Langer, A. Jaklenec, M. Yan. Adv. Healthc. Mater. 5 (2016)2007.
[5] N. Hao, X. Chen, S. Jeon, M. Yan. Adv. Heathcare Mat. 4 (2015) 2797.
[6] S. Xie, S. Manuguri, G. Proietti, J. Romson, Y. Fu, A. K. Inge, B. Wu, Y. Zhang, D. Häll, O. Ramström, M. Yan. Proc. Natl. Acad. Sci. USA 114 (2017), 8464.

Correspondence: Email - mingdi_yan@uml.edu

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Waterways as Reservoirs of Multi-Drug Resistant Enterobacteriaceae (MDR Ent) in a High-Risk Region for MDR Ent Infection in Children

Latania K. Logan[1]*, L. Zhang[2], G. A. Arango-Argoty[2], K. Reme[1], E. Garner[2], S. J. Green[3], S. Dorevitch[3], J. Aldstadt[4], Y. Johnson-Walker[5], M. K. Hayden[1], R. A. Weinstein[1,6], A. Pruden[2]

[1] Rush University Medical Center, Chicago, IL USA; [2] Virginia Tech, Blacksburg, Virginia; [3] University of Illinois at Chicago; [4] University of Buffalo, New York; [5] University of Illinois, Urbana-Champaign, IL; [6] Cook County Health, Chicago, IL, USA

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ABSTRACT

Community-acquired MDR Ent infections are increasing and occurring in people without traditional healthcare exposures. In prior studies we identified neighboring regions in Chicago, Illinois where children living in these regions have a 5-6 times greater odds of MDR Ent infections[1-2]. To prevent community spread of MDR Ent, we need to understand the reservoirs associated with MDR Ent acquisition. A pilot study of surface waters from three recreational waterways (A1-A3) for "incidental contact activities" (e.g. kayaking) and one non-recreational waterway (A4) was conducted. Water samples were collected and filtered using standard EPA methods[3]. Filters were processed for standard bacterial culture, PCR, DNA microarray and shotgun metagenomic sequencing (MGS). Metagenomic DNA was prepared for sequencing on an Illumina NextSeq500 using standard library preparation. Raw reads were submitted to MetaStorm for read mapping and computational profiling of the taxonomy[4]. DeepARG annotated antibiotic resistance genes (ARGs) and MetaCompare ranked sampling sites according to the relative carriage of ARGs and mobile genetic elements (MGEs) by Ent[5-6]. Generally, A4 and A3 were more similar in taxonomy, ARG profiles, and abundances of the corresponding clades and genera within Ent than A2 and A1. Total ARG abundances recovered from the full microbial community were strongly correlated between A4 and A3 (R2=0.97), with a weaker correlation coefficient between A2 and A1 but suggesting they were more similar to each other than to A4 and A3. E. coli numbers (per 100mL water) were highest in A4 (783 Most Probable Number [MPN]) and A3 (200 MPN) relative to A2 (84 MPN) and A1 (32 MPN). In addition, based on MGS analysis (Figure 1) and/or culture, we found concerning ARGs in Ent such as MCR-1 (colistin), Qnr and OqxA/B (quinolones), CTX-M, OXA and ACT/MIR (-lactams), and AAC (aminoglycosides) on MGEs, particularly at sites A4, followed by A3 but also at sites A1 and A2 (e.g. mcr-1 in A2-A4). Our results suggest great potential for ARG mobility and "resistome risk" in regional, including recreational, waterways. Ent and ARG profiles are consistent with the hypothesized concerns that waterways are a source of MDR Ent. Potential ARG sources and exposure risks

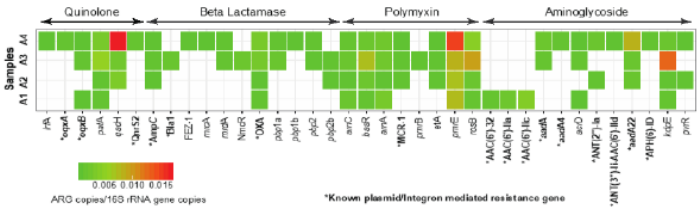

Figure 1: *Enterobacteriaceae* Antibiotic Resistance Profile

References:

[1] L. Logan, R. Medernach, J. Rispens, S. Marshall et al., Ped Inf Dis J 38 (2019).
[2] L. Logan, R. Medernach, N. Domitrovic, J. Rispens et al. Infect Dis Ther (2019).
[3] A. Rompre, P. Servais, J. Baudart, M. De-Roubin et al., J Microbiol Methods 49 (2002) 31-54.
[4] G. Arango-Argoty, G. Singh, L. Heath, A. Pruden et al., PLOS One 11 (2016).
[5] G. Arango-Argoty, E. Garner, A. Pruden, L. Heath et al., Microbiome 6 (2018).
[6] M. Oh, A. Pruden, C. Chen, L Heath et al., FEMS Microbiol Ecol 94 (2018).

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Correspondence: Email - latania_Logan@rush.edu

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Detection of *ndv*B and *tss*C1 genes implicated in biofilm-specific antibiotic resistance in clinical *Pseudomonas aeruginosa* strains

Adriana Silva[1*], Vanessa Silva[1-4], José António Carvalho[5], Ana Paula Castro[5], Soraia Oliveira[1-3], Sara Hermenegildo[1], José Eduardo Pereira[6], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal; [5] Medical Center of Trás-os-Montes e Alto Douro E.P.E., Vila Real, Portugal; [6] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal; Portugal.

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen which represents a threat to human health causing nosocomial infections. This gram-negative bacterium is known to produce robust biofilms that are responsible for adaptation to various environments and resistance against multiple antibiotics [1]. Biofilm is defined as a multicellular community of microorganisms held together by a self-produced extracellular polymeric matrix and the leading cause of hospital-acquired infections that are persistent and very difficult to eradicate. *ndv*B and tssC1 are among the genes that do not influence biofilm formation but are implicated in biofilm-specific antibiotic resistance [2]. Thus, the main aim of this study was to evaluate the presence of *ndv*B and *tss*C1 genes in clinical isolates of *P. aeruginosa*. In this study, a total of 33 *P. aeruginosa* strains were isolated from various human clinical samples from a Portuguese hospital between 2017 to 2019. Antibiotic susceptibility patterns were evaluated by Kirby-Bauer disk diffusion method using 12 antipseudomonal antibiotics according to EUCAST (2018). Molecular detection of *ndv*B and *tss*C1 genes, were amplified by PCR. The antibiotic susceptibility patterns of *P. aeruginosa* isolates demonstrate a high rate of resistance to imipenem (n=33), meropenem (n=21), doripenem (n=20), Cefazolin (n=29), ciprofloxacin (n=19) and piperacillin (n=16). PCR assays showed high prevalence of biofilm-specific antimicrobial resistance genes; 31 isolates harboured the *ndv*B gene and 28 were found to carry *tss*C1. The high presence of *ndv*B and *tss*C1 genes in our study are associated with resistance in biofilm producing *P. aeruginosa* isolates. That genes are related to the problem of antibiotic resistance. Eradication therapy of infections related to bacterial biofilms are becoming a challenge, however, considering the organization, biofilm genes and structure of the *P. aeruginosa* biofilm may assist in the development of novel antibiotic therapy and minimize biofilm infections.

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References:

[1] Saffari et al. 2018. Journal of Medical Microbiology 66: 905–9.
[2] Zhang et al. 2011. Journal of Bacteriology 193 (19):5510–13.

Correspondence: Email - adrianaa.silva95@gmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Carbapenem resistance genes in *Pseudomonas aeruginosa* strains from a Portuguese hospital

Adriana Silva[1*], Vanessa Silva[1-4], José António Carvalho[5], Ana Paula Castro[5], Soraia Oliveira[1-3], Sara Hermenegildo[1], José Eduardo Pereira[6], José L. Capelo[7,8], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal; [5] Medical Center of Trás-os-Montes e Alto Douro E.P.E., Vila Real, Portugal; [6] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal; Portugal; [7] BIOSCOPE Group, LAQV@REQUIMTE, Chemistry Department, Faculty of Science and Technology, NOVA University of Lisbon, Almada, Portugal; [8] Proteomass Scientific Society, Costa de Caparica, Portugal.

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ABSTRACT

Pseudomonas aeruginosa is known as a significant opportunistic pathogen and one of the leading gram-negative organisms associated with common cause of nosocomial infections worldwide, especially in intensive care units [1]. Carbapenems are a group of broad-spectrum β-lactams and they are often use as the last resort for the treatment of serious infections caused by *P. aeruginosa* being considered the most reliable therapeutic options. However, in recent years an increase in prevalence of carbapenem-resistant caused by multiresistant *P. aeruginosa* isolates may significantly compromise their efficacy and represent an emerging challenge to public health, causing higher mortality rates and challenging the current diagnostic approaches [2]. This study aimed to evaluate the presence of carbapenem resistance genes among clinical isolates of *P. aeruginosa*. A total of 33 clinical isolates of *P. aeruginosa* were collected from various human clinical samples from a Portuguese hospital between 2017 to 2019. Isolated organisms were subjected to antimicrobial susceptibility tested by the Kirby-Bauer disk diffusion method using 12 antipseudomonal antibiotics according to EUCAST (2018). The molecular analyses of carbapenems resistant genes will be screened by PCR for detection of bla_{SPM}; bla_{KPC} and bla_{NDM}. All *P. aeruginosa* isolates in this study presented resistant to carbapenems. All isolates displayed resistance to imipenem, 21 isolates showed resistance to meropenem and 20 isolates were resistance to doripenem. In addition, these isolates are multidrug resistant as they were resistant to three or more classes of antimicrobials, such as cephalosporin, fluoroquinolones and penicillin. The prevalence of carbapenem genes was relatively high in the current study; bla_{SPM} was identified in 31 isolates, 6 isolates harbored the bla_{KPC} and 7 the bla_{NDM} gene. In conclusion, our study highlights the increasing carbapenem resistance in *P. aeruginosa* and despite efforts to control this resistance, carbapenemase-encoding genes (SPM, KPC and NDM) are already widespread and threat to public health. One of the situations that can contribute to this resistance and emergence phenomenon is antibiotic selective pressure. Therefore, it is important to develop and implement alternative approaches to avoid the dissemination of resistant isolates

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References:

[1] Vanesa Estepa, et al. Enfermedades Infecciosas y Microbiologia Clinica 35 (3) (2017). SEGO:141–47.
[2] J. Vanegas, et al. Journal of Clinical Microbiology 52 (11) (2014):3978–86.

Correspondence: Email - adrianaa.silva95@gmail.com

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Antibiotic resistance characterization of *Enterococcus* spp. strains isolated from fish species used in sushi preparation

Anicia Gomes[1-3]*, José Pedro Sampaio[1-3], Vânia Santos[1-3], Carla Andrea Alonso[4], Laura Ruiz-Ripa[4], Sara Ceballos[4], Gilberto Igrejas[1,2,5], Carmen Torres[4], Patrícia Poeta[3,5]

[1] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [4] Biochemistry and Molecular Biology Unit, University of La Rioja, Logroño, Spain; [5] LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Portugal.

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ABSTRACT

The increase of antimicrobial resistance results from the abusive use of antibiotics in human and animal health and animal production over the years, exerting selective pressure on the microorganisms and favouring the emergence and dissemination of resistant bacteria. *Enterococcus* spp. are commensal bacteria of the intestinal microbiota, extremely versatile, and can survive in a wide diversity of conditions, becoming increasingly reported as an opportunistic pathogen[1]. Little is known about the enterococcal species diversity and distribution of resistance determinants in enterococci isolated from fish samples. The aimof this study was to evaluate the prevalence, phenotype/genotype of antibiotic resistance and bacteriocin production of enterococci isolated from fish samples for human consumption. We also determined the VRE rate among the samples, vancomycin resistance mechanisms, type of Tn*1546*, the presence of virulence genes and the genetic lineages of VRE. 150 samples were analysed and 63 enterococci were recovered when inoculated in Slanetz-Bartley, with the following species detected: *E. faecium-E. faecalis* (85.7%) and *E. hirae-E. gallinarum-E. mundtii* (14.3%). MDR phenotypes were found in 15.2% of enterococci. VSE strains showed high rates of resistance to tetracycline (40.7%, mostly by *tetM*), erythromycin (33.9%, mostly by *ermB*) and kanamycin (35.6%). Gentamicin-chloramphenicol resistance was the lowest frequency detected (1.7%). The *aac(6')-Ie-aph(2")-Ia* gene was detected in one high-level-gentamicin-resistant *E. faecium* of the new lineage ST1396. Sixty-per-cent of enterococci produced antimicrobial substances against different indicator bacteria and the *entA* was the most prevalent gene. VRE was detected in 4 samples (2.7%), that is, 3 *E. faecium* and 1 *E. faecalis*, all with *vanA* genotype. The *E. faecium* VRE were ascribed to ST139 (CC5), and the *E. faecalis* to ST16 (CC16). VRE isolates were MDR and carried the genes *tetM, tetL, tetK, ermB, aac(6')-Ie -aph(2")-Ia* and/or cat_{pC223}. The *hyl* and *gelE* genes were absent, but the *esp* gene was identified. VRE strains showed the standard Tn*1546* structure and the *vanA* gene could not be transferred by conjugation. These results indicate that fish species for human consumption are contaminated with enterococci with relevant antimicrobial resistances, such as VRE with the vanA genotype, among others, with potential implication in public health. The data obtained in this study are important to alert for the prudent use of antibiotics and warn the population about the risks of consumption of raw/undercooked products.

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References:

[1] C. Torres, C. A. Alonso, L. Ruiz-Ripa, R. León-Sampedro, R. del Campo, T. M. Coque. Microbiol Spectrum 6 (2018).

Correspondence: Email - aniciagomes88@gmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Analysis of virulence genes and agr types among methicillin-resistant *Staphylococcus aureus* from infected diabetic foot ulcers

Francisco Almeida[1*], Vanessa Silva[1-4], José Eduardo Pereira[5], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal; [5] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal; Portugal.

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a major threat to public health, classified by World Health Organization as one of the highest priority world pathogenic bacteria and responsible for a big part of all deaths caused by antibiotic resistant microorganisms [1]. Besides being multi-resistant it also has a wide spectrum of virulence factors regulated by accessory genes bound to tissue invasion, surface adhesion, evasion from host immune system and commonly associated with diseases. This study aims to characterize virulence factors and accessory gene regulator (*agr*) among MRSA isolated from infected diabetic foot ulcers. The 28 MRSA isolates were tested for the presence of haemolysis-a and b genes (*hla* and *hlb*), toxic shock syndrome toxin gene (*tst*), exfoliative toxin a and b genes (*eta* and *etb*) using specific primers and conditions [2]. All isolates were characterized by *agr*-typing using specific primers [3]. The virulence genes detected were *hla* (n=26), *hlb* (n=13), *tst* (n=5) and *eta* (n=4). The MRSA strains belonged mainly to *agr*-type I (42.8%), followed by *agr*-type II (35.7%) and *agr*-type III (17.9%). Only one isolate was *agr*-negative and *agr* IV was not detected in this study. All isolates belonging to *agr*-type I harboured the *hla* gene and none encoded the *tst*. Furthermore, the presence of *etb*-carrying strains was not found. The virulence factors are strongly related to *agr* phylogeny. *eta* and *etb* genes are linked to type IV group while the *tst* is preferentially carried by *agr*-III strains [4]. It is also known that types I and II play a key role on regulating haemolysins [4]. Infections by multiresistant bacteria on this type of ulcers make treatment complex and longstanding therefore expensive, and which sometimes leads to the amputation of the lower limb. A better understanding of the virulence genes and *agr*-types is crucial in the development of new drugs and treatments effective against these organisms.

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References:

[1] E. Tacconelli, E. Carrara, A. Savoldi, et al, The Lancet Infectious Diseases. (2018) 318-327.
[2] F. Yu, Y. Liu, J. Lv, et al. J Infect Dis. 19 (2015) 614–22.
[3] B. Shopsin, B. Mathema, P. Alcabes, et al. J Clin Microbiol. 41 (2003) 456.
[4] S. Jarraud, C. Mougel, J. Thioulouse, et al. Infection and Immunity. 70 (2002) 631-641;

Correspondence: Email - franciscoal95@hotmail.com

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High frequency of ESBL- *E. coli* producers in pets in Portugal with detection of ST131 clone carrying different variants of CTX-M genes

Isabel Carvalho[1-4*], Paulo Pimenta[5], Rita Cunha[6], Carla Martins[7], Gilberto Igrejas[2-4], Carmen Torres[8], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, Universidade de Trás-os-Montes e Alto Douro, Portugal; [2] Department of Genetics and Biotechnology, UTAD, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, UTAD, Vila Real, Portugal; [4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisbon, Portugal; [5] Hospital Veterinário de Trás-os-Montes, Vila Real, Portugal; [6] Hospital Veterinário de São Bento, Lisboa, Portugal; [7] Clínica Veterinária do Vouga, Sever do Vouga, Portugal; [8] Area Biochemistry and Molecular Biology, University of La Rioja, Logroño, Spain.

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ABSTRACT

Escherichia coli is frequently implicated in community and hospital-associated infections in humans and companion animals [1]. The increasing prevalence of infections with organisms producing extended spectrum β-lactamases (ESBL, particularly those of the CTX-M type) is threatening the future of the β-lactam drugs [2]. The *E. coli* ST131 is an epidemic clone that has been frequently associated to CTX-M-15 [3]. The objective of this work was to determine the carriage rate of ESBL-producers E. coli in pets in Portugal, and the type of enzymes implicated. Fecal samples were recovered from 501 apparently healthy pets (361 dogs and 140 cats) during april-august 2017, and they were seeded on MacConkey agar supplemented with cefotaxime (2 µg/ml). Antimicrobial susceptibility was performed by disk-diffusion test (CLSI, 2017). The presence of blaCTX-M (different groups) were tested by PCR/sequencing. Furthermore, phylogenetic groups were determined and the ST131 clone was identified by specific-PCR. ESBL producing *E. coli* were detected in 8.6% of cats and in 13% of the tested dogs. Most of ESBL producing E. coli of cats (11/11) and dogs (45/47) carried variants of the CTX-M-type gene, mostly of the group 1 (CTX-M-1, CTX-M-9,CTX-M-14, CTX-M-15, CTX-M-27, CTX-M-32 and CTX-M-55). ESBL-positive isolates in cats were mostly ascribed to phylogenetic group B2 while dogs were to A+B1 phylogenetic group (74.5%). Moreover, CMY-2-producing isolates were detected in three animals (two dogs and one cat). The clone ST131-B1 was detected in three isolates of cats (with the genes of CTX-M-1, CTX-M-15, CTX-M-27) and in two of dogs (CTX-M-15). Our results suggest the potential zoonotic role of dogs and cats in the transmission to humans of ESBL in the household environment, highlighting the presence of the ST131-B2 clone.

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References:

[1] A. Derakhshandeh, et al. Microb Pathog, 116 (2018) 241-245.
[2] S. Karanika, et al. Clin Infect Dis. 63 (2016) 310-8.
[3] C. A. Alonso, et al. Lett Appl Microbiol. 64 (2017) 318-334.

Correspondence: Email - isabelcarvalho93@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Phenotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) of human osteomyelitis

Mário Bezerra[1-3*], Bárbara Lima[1-3], Vanessa Silva[1-4], José Eduardo[1,5], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [4] LAQV-REQUIMTE, Chemistry Department, Faculty of Science and Technology, University NOVA of Lisbon, Caparica, Portugal;[5] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal.

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ABSTRACT

Osteomyelitis is a clinical condition based on a general bone infection. This condition can be hematogenous or exogenous which allows the bacterial contamination that leads to an osteomyelitis [1]. Antibiotic resistance is emerging as a dangerous public health concern due to the decreasing number of therapies available. Staphylococcus aureus is a pathogen associated to high mortalitywhich can induce infections in several tissues of the human body and is the most common cause of acute and chronic hematogenous osteomyelitis in adults and children. Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most known microorganisms related to several clinical conditions associated with antibiotic resistance [2]. Besides, MRSA is considered a danger nosocomial agent. So, this study aimed to study the antibiotic resistance of MRSA strains isolated from human osteomyelitis. Osteomyelitis samples were seeded onto Oxacillin-Resistance-Screening-Agar-Base plates with 2 mg/L of oxacillin for the isolation of MRSA. The antimicrobial susceptibility of the isolates was tested by the Kirby-Bauer disk diffusion method against fifteen antimicrobial agents: cefoxitin (30 µg), oxacillin (1 µg), penicillin (10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), tobramycin (10 µg), kanamycin (30 µg), gentamicin (10 µg), clindamycin (2 µg), fusidic acid (10 µg), tetracycline (30 µg), linezolid (10 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1,25-23,75 µg) and mupirocin (5 µg) and according to EUCAST (2018), with the exception of kanamycin that followed the CLSI guidelines (2017). Forty-one MRSA isolates were recovered from osteomyelitis. All MRSA showed resistance to cefoxitin and oxacillin. Resistance to penicillin (n=40), ciprofloxacin (n=38), erythromycin (n=32), tobramycin (=5), kanamycin (n=4), gentamicin (n=3), clindamycin (n=3), fusidic acid (n=3) and tetracycline (n=2) was also detected in this study. None of the isolates showed resistance to linezolid, chloramphenicol, trimethoprim-sulfamethoxazole and mupirocin proving the multi-resistant character of the isolates. Over time, we have seen an increasing of antibiotic resistance associated to MRSA which has been describe by the World Health Organization (WHO) as highly pathogenic agents. Due to the clinical significance of this kind of the resistance, it has become a lot harder to apply, in an efficient way, antibiotics to overcome the osteomyelitis.MRSA bone infections may be persistent which could lead to serious effects on the healing process and morbidity.

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References:

[1] Siqueira, E. G. M., et al. Veterinary Quarterly. 34 (2014): 201-204.
[2] Sousa, Margarida, et al. Veterinary microbiology. 171 (2014): 436-440.

Correspondence: Email - mariojbezerra02@gmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Dalbavancin as a drug to combat resistant Gram-positive bacteria in biofilms and osteomyelitis in rats

Vanessa Silva[1-4], Sofia Antão[5], João Guimarães[5], Justina Prada[1,6], Isabel Pires[1,6], Luís Maltez[1,7], José Eduardo Pereira[1,7], Gilberto Igrejas[2-4], Patrícia Poeta[1,4*]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; 4 Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal; [5] Angelini Farmacêutica Lda, medical department Angelini, Medical Department, Dafundo, Portugal; [6] CECAV, Vila Real, Portugal; [7] ???

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is resistant to the broad-spectrum of antibiotics, including penicillins and cephalosporins, and have the ability to cause serious infections. Orthopedic-related infections are very difficult to treat and involve surgical procedures and prolonged antibiotherapy. Osteomyelitis caused by MRSA is one of the most difficult and challenging bone infections to treat [1]. Besides, MRSA strains are often responsible for chronic infections due to their ability to produce biofilms, in particular, on abiotic surfaces, such as, medical implants [2]. MRSA infections are usually treated with vancomycin and linezolid, however, when the MRSA strains are multiresistant the optional therapies fail. Therefore, we aimed to investigate the efficacy of dalbavancin as a new therapeutic agent to treat MRSA osteomyelitis and to eradicate bacterial biofilm infections related to medical implants. One MRSA strain isolated from human osteomyelitis was used in this study to promote the development of osteomyelitis in rat tibia and biofilm formation on a stainless-steel screws surface. Seventy-eight Wistar rats were divided into 6 groups: osteomyelitis control group (no treatment), osteomyelitis group 1 (7 days of treatment), osteomyelitis group 2 (14 days of treatment), biofilm control group (no treatment), biofilm group 1 (7 days of treatment), biofilm group 2 (14 days of treatment). Dalbavancin (10 mg/kg/day) was administered intraperitoneally in all treatment groups. The osteomyelitis was induced by drilling a hole in the tibia and adding 10 µL of MRSA inoculum. The 1.5 mm screws provided with biofilms were placed on the proximal tibia under general anesthesia. Bacterial loads of both the tibia and the implant were quantified using plate count agar. The high number of colony forming units per milliliter (cfu/ml) present in both control groups indicated a well-established infection. Dalbavancin use correlated with a significant reduction in osteomyelitis and in implant associated infection, with a lower MRSA cfu count compared with the control group. A significant reduction of cfu/ml was observed in the osteomyelitis group 7 days after treatment, and in the group treated for 14 days there was no signs of infection. A reduction in the number of cfu was also detected in the biofilm groups, nevertheless, after 14 days of treatment the infections was not totally eradicated. Dalbavancin seems to have a total antimicrobial effect on MRSA osteomyelitis, nevertheless, and although 14 days after the treatment there was a marked decrease in cfu number, biofilm-induced infection still prevailed. Further studies should be carried out to evaluate the potential of dalbavancin in the treatment of bone and orthopedic implant associated MRSA infections.

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References:

[1] A. M. Tuzuner-Oncul, C. Ungor, U. Dede, R. S. Kisnisci. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107 (2009).
[2] M. R. Kiedrowski, A. R. Horswill. Ann N Y Acad Sci. 1241 (2011) 104-121.

Correspondence: Email - ppoeta@utad.pt

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Prevalence of biofilm-related genes in methicillin-resistant *Staphylococcus aureus* isolated from patients with septicaemia

Sara Hermenegildo[1*], Vanessa Silva[1-4], Adriana Silva[1], José António Carvalho[5], Ana Paula Castro[5], José Eduardo Pereira[6], José L. Capelo[7,8], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal; [5] Medical Center of Trás-os-Montes e Alto Douro E.P.E., Vila Real, [6] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal; Portugal; [7] BIOSCOPE Group, LAQV@REQUIMTE, Chemistry Department, Faculty of Science and Technology, NOVA University of Lisbon, Almada, Portugal; [8] Proteomass Scientific Society, Costa de Caparica, Portugal

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ABSTRACT

The ability of *S. aureus* to form biofilms on biomaterials is probably the major contributing factor to wound infections and on catheters, shunts, implants, among other. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causative agents of septicaemia in Portugal. MRSA is able to form biofilm not only in physical structures but also in tissues which leads to various clinical manifestations by patients, such as bacteraemia, endocarditis, osteomyelitis as well as other severe chronic infections [1]. Thus, this study aims to characterize the potential for biofilm production and identify genes responsible for the formation of biofilms. Eleven presumptive MRSA isolates were recovered from patients with septicaemia admitted to the local hospital between 2016 and 2019. The confirmation of *Staphylococcus* species and resistance to methicillin were carried out by multiplex PCR of the genes 16S, *nuc* and *mecA*. The potential for biofilm production was determined by the Congo Red Agar (CRA) assay. The biofilm-related genes were studied in MRSA strains by PCR using specific primers and conditions. All isolates were Staphylococcus aureus resistant to methicillin. Seven isolates showed slime production on Congo Red agar (CRA). Biofilm-related genes were expressed in at least 8 isolates, with exception of *fnbA, clfA* and *fib* genes as follows: *bbp*=8, *icaB*=9, *cna*=10, *ebps*=11, *icaD*=11, *clfB*=11 and *eno*=11. This genotypic characterization method confirmed the formation of biofilms in slime-producing strains in CRA. Biofilm-producing MRSA have serious clinical implications and it is difficult to eradicate these due to the increased tolerance to antimicrobials. Our results illustrated the presence of several genetic markers involved in the production of biofilm in strains of MRSA. It is, thus, extremely important to know the characteristics of each strain that causes the infection, as well as, the patterns of antimicrobial susceptibility in order to assist in the choice of the best antimicrobial therapy and the correct elimination of biofilm [2].

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References:

[1] T. Oyama, M. Miyazaki, M. Yoshimura, T. Takata, H. Ohjimi, S. Jimi. Toxins 8 (2016) 198
[2] S. O. Moghadam, M. Pourmand, F. Aminharati. J Infect Dev Ctries. 8 (2014) 1511-1517.

Correspondence: Email - sara-1603@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Antibiotic resistance in methicillin-resistant *Staphylococcus aureus* isolated from patients with septicaemia

Sara Hermenegildo[1*], Vanessa Silva[1-4], Adriana Silva[1], José António Carvalho[5], Ana Paula Castro[5], José Eduardo Pereira[6], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal; [5] Medical Center of Trás-os-Montes e Alto Douro E.P.E., Vila Real, Portugal; [6] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal.

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) represents one of the major nosocomial agents causing septicaemia and is highly resistant to almost all beta-lactam antibiotics. MRSA is responsible for causing a variety of human infections. Patients with severe MRSA infection, without adequate therapy in time, are expected to have a low life expectancy [1]. Thus, this study aims to characterize antibiotic resistance in MRSA isolated from septicaemia strains and identify virulence factors. Eleven MRSA isolates were recovered from patients with septicaemia admitted to the local hospital between 2016 and 2019. The susceptibility of these isolates was tested by the Kirby-Bauer disk diffusion method against 13 antimicrobial agents and according to EUCAST (2018) standards. The antimicrobial agents used were penicillin (10 un), gentamicin (10 µg), mupirocin (200 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), fusidic acid (10 µg), clindamycin (2 µg), linezolid (10 µg), tobramycin (10 µg), kanamycin (30 µg), trimethroprim-sulfamethoxazole (1.25-23.75 µg) and tetracycline (30 µg). The resistance and virulence genes were studied in MRSA strains by PCR using specific primers and conditions. All isolates carried the *nuc*, 16s and *mecA* genes which confirms the MRSA strains. All MRSA strains showed resistance to at least 3 different classes of antibiotics and, therefore, were considered multiresistant. The isolates showed resistance to penicillin (n=11), oxacillin (n=11), cefoxitin (n=11), ciprofloxacin (n=11), erythromycin (n=8), fusidic acid (n=1) and clindamycin (n=1). This was confirmed by the presence of the genes: *ermA, ermC, mphC, blaZ, msrA/B* and *vgaE*. The virulence genes found were as follows: hla (n=11), hlb (n=6) and etA (n=7). The prevalence of MRSA has been increasing in Portugal. These strains are implicated in septicaemias causing high morbidity and mortality. Thus, the characterization of this type of strains may represent a promising approach for developing more targeted treatment strategies for persistent S. aureus [2].

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References:

[1] A. E. Simor, L. Pelude, G. Golding, R. Fernandes, E. Bryce, C. Frenette, K. Weiss. Infect Control Hosp Epidemiol. 37 (2016) 390–397;
[2] G. Taylor, K. Bush, J. Leal, E. Henderson, L. Chui, M. Louie. J Hosp Infect. 89 (2015) 132–135.

Correspondence: Email - sara-1603@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Detection of TEM-, SHV- and CTX-M-type beta-lactamase production in *Escherichia coli* from processed meat

Soraia Oliveira[1-4*], Vanessa Silva[1-4], Adriana Silva[1], Susana Correia[1-4], José Eduardo[1,5], José L. Capelo[6,7], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Veterinary Science Department, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [4] LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Portugal; [5] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal; [6] BIOSCOPE Group, LAQV@REQUIMTE, Chemistry Department, Faculty of Science and Technology, NOVA University of Lisbon, Almada, Portugal; [7] Proteomass Scientific Society, Costa de Caparica, Portugal.

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ABSTRACT

There is a worldwide increase in infections caused by Gram-negative bacteria producing extended-spectrum β-lactamases (ESBL) [1], being these most commonly produced by *Escherichia coli* [2]. The presence of ESBL-producing *E. coli* in food-producing animals and contamination of retail meat may contribute to increased incidences of these infections in humans, leading to a public health problem [2]. Thus, we aimed to detect ESBL production, antibiotic susceptibility patterns, and TEM-, SHV- and CTX-M-encoding genes, in *E. coli* isolates arising from processed meat. Different processed meat samples obtained from several commercial establishments were seeded in Levine EMB agar plates supplemented with cefotaxime (2 µg/mL). Susceptibility to 16 antimicrobial agents was performed by the disk diffusion method according to CLSI criteria [3]. Detection of the ESBL phenotype was performed by the combined disk method. A total of 15 ESBL-producing *E. coli* isolates were obtained from hamburgers (N=7), meatballs (N=6) and minced meat (N=2). All isolates shown resistance to cefotaxime and ampicillin and were susceptible to tobramycin, amikacin and imipenem. High resistance was detected for tetracycline (N=14), aztreonam (N=10), amoxicillin + clavulanic acid (N=8) and ceftazidime (N=7). Additionally, resistance to trimethoprim-sulfamethoxazole (N=4), streptomycin (N = 3), chloramphenicol (N = 3), nalidixic acid (N = 2) and ciprofloxacin (N = 2) was observed. The *bla*TEM gene was detected in six isolates (4 from hamburgers and 2 from minced meat), and one isolate from meatballs harboured the *bla*SHV gene. A total of 11 isolates were CTX-M positive, with one isolate recovered from hamburgers showing the combination of both *bla*TEM and *bla*CTX-M genes. The dissemination of CTX-M-positive bacteria considerably alters the way community-acquired infections are treated and limits the oral antibiotics that can be administered [4]. *E. coli* is one of the most common causes of food and water-borne human infections worldwide [5]. Therefore, the prevalence of β-lactamase producing *E. coli* in retail meat constitutes a major public health concern.

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References:

[1] M. A. L. Hall, C. M. Dierikx, J. C. Stuart, G. M. Voets, M. P. Van Den Munckhof. (2011).
[2] T. M. Coque, F. Baquero, R. Canton. (1983).
[3] CLSI, Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fourth Informational Supplement, CLSI docum, Wayne, PA: Clinical and Laboratory Standarts Institute, 2017.
[4] J. D. D. Pitout, P. Nordmann, K. B. Laupland, L. Poirel. J. Antimicrob. Chemother. 56 (2005) 52–59.
[5] S. M. Turner, A. Scott-Tucker, L. M. Cooper, I. R. Henderson. FEMS Microbiol. Lett. 263 (2006).

Correspondence: Email - soraia_oliveira_90@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Antimicrobial Resistance and Genotypic Characterization of Vancomycin-Resistant Enterococci isolated from Hamburgers and Minced meat

Soraia Oliveira[1-4*], Vanessa Silva[1-4], Adriana Silva[1], Susana Correia[1-4], José Eduardo[1,5], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Veterinary Science Department, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [4] LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Portugal; [5] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal.

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ABSTRACT

Enterococci are frequently associated with various infections and diseases, both in humans and animals. The widespread use of antibiotics in animal production has implications in human health, being accessible through food products. Thus, we aimed to identify vancomycin-resistant enterococci (VRE) from processed meat and investigate antimicrobial resistance (AMR) and virulence determinants. Different processed meat samples obtained from several commercial superficies were seeded in Slanetz-Bartley agar supplemented with vancomycin (4 µg/mL). Species identification was confirmed by PCR [1]. Susceptibility for 11 antimicrobial agents was performed by disk diffusion according to CLSI [2]. High-level resistance was evaluated for aminoglycosides. Genes encoding AMR and virulence were analysed by PCR. Vancomycin resistance mechanisms were analysed using specific primers for the *vanA, vanB, vanC-1, vanC-2/3,* and *vanD* genes [3]. Isolates were identified as *E. faecium* (n=14), *E. durans* (n=1), and *E. gallinarum* (n=3). All strains showed resistance to three or more antimicrobials, in addition to vancomycin. Higher incidence of resistances was observed for quinupristin-dalfopristin (n=18), erythromycin (n=16) and tetracyclin (n=15). The *vanA* gene was identified in all strains, except for all *E. gallinarum* strains (in which the *vanC-1* gene was detected). Most isolates were tetracycline-resistant: eight with the combination *tet(M)+tet(L)+tet(K)* and five with the combination *tet(M)+tet(L).* Additionally, the *E. durans* strain exhibit the *ant(6)-I+erm(B)* genes and the combination of *aac (6)'aph(2)"+erm(B)* genes were detected in both *E. faecium* (n=1) and *E. gallinarum* (n=1) species. Virulence gene combinations were also detected: *hyl+cylLS* in *E. faecium* (n=1), *hyl+cylLS+cpd* in *E. gallinarum* (n=1) and *hyl+cpd+cylA+cylB+cylM+cylLL* in *E. faecium* (n=1). The *hyl* gene was detected in all species. Our study suggests that meat food plays a potential role as reservoirs of resistance determinants, prompting the need to undertake epidemiological and molecular studies to evaluate the mobility of these genes. The consumption of contaminated meat may be associated with the spread and colonization of VRE in humans and for this reason could represent a public health concern.

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References:

[1] P. Poeta, D. Costa, Y. Sáenz, et al. Zoonoses Public Health 52 (2005)396–402.
[2] CLSI, Performance Standards for Antimicrobial Susceptibility Testing: 24th Informational Supplement, CLSI docum, Wayne, PA: Clinical and Laboratory Standarts Institute, 2017.
[3] C. Torres, C. Tenorio, A. Portillo, et al. Microb. Drug Resist. 9 (2003)47–52.

Correspondence: Email - soraia_oliveira_90@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Identification of multiresistant methicillin-resistant *Staphylococcus aureus* (MRSA) in wild hares (*Lepus granatensis*) from Portugal

Vanessa Silva[1-4*], Adriana Silva[1-3], Soraia Oliveira[1-4], José Eduardo Pereira[1,5], José L. Capelo[6,7], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [4] Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Caparica, Portugal; [5] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal; Portugal; [6] BIOSCOPE Group, LAQV@REQUIMTE, Chemistry Department, Faculty of Science and Technology, NOVA University of Lisbon, Almada, Portugal; [7] Proteomass Scientific Society, Costa de Caparica, Portugal.

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of infections both in humans and animals [1]. The presence of MRSA in food and wild animals is considered a public health problem since they may represent an important and potential route of transmission between animals and humans [2]. The presence of these strains in humans, pets and livestock animals have been widely investigated, nevertheless, there is still little information in the prevalence of MRSA strains in wild animals. Therefore, the present study was undertaken to investigate the occurrence of MRSA strains in wild hare. Eighty-three wild hares (*Lepus granatensis*) were captured in the north of Portugal by hunting associations during the hunting season from September to December 2018. Samples were collected from both nostrils, buccal mucosa and perianal skin using only one swab per animal. The swabs were aseptically placed into tubes containing 5 mL of brain heart infusion broth supplemented with 6.5% of NaCl and incubated at 37ºC for 24h. Then, 150 µL of inoculum was seeded onto ORSAB plates supplemented with 2 mg/L of oxacillin for MRSA isolation. The presumptive MRSA strains were identified by Gram staining, DNase and catalase, and by multiplex PCR. The susceptibility of the isolates was tested by the Kirby-Bauer disc diffusion method against 14 antimicrobial agents and according to EUCAST (2018) standards, with the exception of kanamycin that followed the CLSI guidelines (2017). The presence of resistance genes was studied by PCR using specific primers and conditions. From the 83 samples, 4 (4.8%) MRSA strains were isolated. All strains presented resistance to penicillin and cefoxitin and two harboured the *blaZ* gene. Resistance to macrolides and lincosamides was found in all strains, among the strains the *ermC* (n=3), *ermT* (n=3) *ermB* (n=2), *mphC* (n=2) and *ermA* (n=1) genes were detected. All isolates harboured the *dfrA* gene encoding resistance to trimethoprim-sulfamethoxazole. One MRSA strain showed resistance to gentamycin and presented the *aac(6')-Ie-aph(2")-Ia* gene. The prevalence of MRSA strains in wild hares was low, nevertheless, the strains found in these animals were multiresistant. Even though wild hares do not contact directly with antibiotics these animals can be colonized by resistant bacteria and act as a reservoir of antimicrobial resistant bacteria, in particular MRSA strains. Antibiotic resistance can be transmitted between wild animals and humans through the consumption of contaminated meat or through the environment. Therefore, it is important to investigate the prevalence antibiotic resistance strains and roots of transmission to ascertain the risk of colonization of humans and animals.

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References:

[1] J. S. Weese. ILAR J. 51 (2010) 233-44.
[2] A. Traversa, G. R. Gariano, S. Gallina, et al. Food Microbiology. 52 (2015) 154-158.

Correspondence: Email - vanessasilva@utad.pt

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Linezolid resistant *cfr*-positive methicillin-resistant *Staphylococcus aureus* isolated from infected diabetic foot ulcers

Vanessa Silva[1-4*], Francisco Almeida[2], Soraia Oliveira[1-4], Vera Manageiro[5,6], Eugénia Ferreira[5,6], José Eduardo Pereira[1,7], Manuela Caniça[5,6], Gilberto Igrejas[2-4], Patrícia Poeta[1,4]

[1] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [4] LAQV-REQUIMTE, University NOVA of Lisboa, Caparica, Portugal; [5] National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRL-AMR/HAI), Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge, Lisbon, Portugal; [6] Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, Oporto University, Oporto, Portugal; [7] Centre of Studies of Animal and Veterinary Sciences, Vila Real, Portugal.

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worrisome microorganism resistant to almost all beta-lactams and frequently carries resistance to other major antibiotic classes. Portugal has one of the highest rates of MRSA in Europe being of great concern at hospital level since about 39.2% of the *S. aureus* isolates with invasive origin in Portugal are methicillin-resistant [1]. Linezolid is used as an important resort in complicated soft tissue infections in multidrug-resistance MRSA strains. However, despite the prevalence of linezolid-resistant MRSA strains has remained overall low, in the past few years, resistance to linezolid have been reported among human patients worldwide imposing a public health concern, in particular when associated with the *cfr* gene [2]. Therefore, we aimed to characterize linezolid-resistance mechanism in MRSA isolates recovered from infected diabetic foot ulcers, as well as, to analyse their genetic lineages. Overall, samples were collected from 45 type 2 diabetic patients with infected foot ulcers. The isolates were seeded onto Oxacillin-Resistance-Screening-Agar-Base (ORSAB) with 2 mg/L of oxacillin for the isolation of MRSA strains. The minimum inhibitory concentrations (MIC) of linezolid was further investigated using E-test strips. Susceptibility to antibiotics was tested by the Kirby-Bauer disk diffusion method against 14 antibiotic agents and interpreted according EUCAST guidelines. The presence of resistance and virulence genes were studied by PCR and sequencing. All isolates were characterized by agr, spa SCCmec and multilocus sequence typing. Among the 45 samples 28 MRSA isolates were detected, and between them 3 showed resistance to linezolid, with MICs varying from 8 to 16 mg/L; the 3 isolates carried the cfr gene, and showed resistance to penicillin and cefoxitin, and harboured the blaZ gene. Phenotypic resistance to tetracycline (n=2), ciprofloxacin (n=2), erythromycin (n=2), clindamycin (n=2), fusidic acid (n=2), gentamicin (n=2), and trimethoprim-sulfamethoxazole (n=2) was also found. All isolates showing resistance to tetracycline harboured the tetL and tetO genes, one isolate also harboured the tet(K) gene. Isolates presenting resistance to trimethoprim-sulfamethoxazole harboured the dfrA and dfrK genes, and the dfrG gene was only found in one isolate. Linezolid-resistant MRSA isolates were assigned to the pandemic nosocomial clones ST22-IV/t747 (EMRSA-15), ST105-II/t002 (New York/Japan related) and ST8-IV/t1476 (USA300). The detection of 3 clinical MRSA strains carrying the cfr gene which encodes resistance to linezolid is alarming since at our knowledge this gene had not been yet found circulating among human S. aureus population in Portugal. Besides, in this study, linezolid resistant strains are associated with the pandemic clones which is a cause for concern.

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References:

[1] Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) (2017). Stockholm: ECDC; 2018
[2] Antonelli A, D'Andrea MM, Galano A, et al. J Antimicrob Chemother (2016); 71: 2349–51.

Correspondence: Email - vanessasilva@utad.pt

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MRSA-CC398 detection of infection in pigs and piglets carcasses discarded at slaughterhouse level in Portugal

Vânia Santos[1,2,6*], Anícia Gomes[1,2], Laura Ruiz-Ripa[6], Olouwafemi Mistourath Mama[6], Carolina Sabença[1-3], Margarida Sousa[1-4], Vanessa Silva[1-3,5], Telma Sousa[1-3], Madalena Vieira-Pinto[3,4], Gilberto Igrejas[1,2,5], Carmen Torres[6], Patrícia Poeta[3,5]

[1] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [4] Veterinary and Animal Science Research Center (CECAV), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [5] Associate Laboratory for Green Chemistry-LAQV, Chemistry Department, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Caparica, Portugal; [6] Area of Biochemistry and Molecular Biology, University of La Rioja, Logroño, Spain.

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) are resistant to most beta-lactam antibiotics. In 2005, a distinct clone (MRSA CC398) was found in pigs and people in contact with pigs in the Netherlands. Since then, several countries have detected MRSA CC398 in pig herds and other livestock, and its presently regarded an important zoonotic agent [1]. The aim of this study was to determine if MRSA CC398 could be the cause in skin and white parts infections in pigs. To do so, we analysed pig carcasses from three slaughterhouses in Portugal, that have been cut off from the food chain due to signs of infection. For this, 141 samples were taken from the infection focus, and were inoculated in Mannitol Salt agar and ORSAB for isolation of S. aureus and MRSA, respectively. The strains were identified by MALDI-TOF and proceeded to molecular characterization of S. aureus strains (spa, CC398, SCCmec), the study of resistance genes to antibiotics and virulence, the presence of scn gene from Immune Evasión Cluster (IEC) system and prophages, by PCR and sequencing. In 28 of 141 samples analysed, S. aureus was detected (20%), 21 of them corresponded to abscesses in piglets, and 7 to samples from osteomyelitis. Most of the strains were MRSA and 6 of 28 were MSSA. All MRSA strains were typed within the CC398 lineage and three spa types were identified (t011, t108 and t1451), being the most frequent t1451. The MRSA-CC398 strains contained SCCmec V (spa t011 and t108 strains) and SCCmec Iva (spa t1451 strains). The MSSA strains were typed as spa-t1491-ST1-CC1. All the strains obtained in this study were negative for the IEC system. They were also negative for eta, etb and cna genes and the Panton-Valentine leukocidin (PVL). In terms of prophages, all the MSSA obtained the same profile (Sa2Sa7), whereas MRSA strains showed variation between Sa1 and Sa2. The MRSA strains presented the following resistance phenotypes/genotypes: tetracycline (100%, tetM, tetk and tetL), erythromycin (54.5%, ermC and msr(A)), clindamycin (68.2%, vgaB and InuB), gentamicin (50%, aac(6')-aph(2")-Ia), cefoxitin-oxacillin (100%, mecA), chloramphenicol (40.9%, fexA, catpC221, catpC223 and catpC194), and trimethoprim-sulfamethoxazole (95%, dfrA and dfrG). Related to virulence profile, the MRSA obtained the same in all the strains (hla, hlb, hld and hlg), where the same happened in the MSSA strains (hla, hlb, hld and hlgv). Our results are in line with previously studies where MRSA-CC398 is most common in pigs [2,3], which leads to a relevant issue with regard to food safety and consumer protection [4]. So it's important to prevent their dissemination on the farm and along the food chain, since these strains could harm the veterinarian or the technician inspectors making them MRSA carriers and the final consumer if the carcasses aren't well incinerated.

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References:

[1] Broens, E. M. et al. Prev. Vet. Med. 98, 182–189 (2011).
[2] Schwarz, S., Kadlec, K. & Strommenger, B. J. Antimicrob. Chemother. 61, 282–285 (2008).
[3] de Neeling, A. J. et al. Vet. Microbiol. 122, 366–372 (2007).
[4] Feßler, A. T. et al. Appl. Environ. Microbiol. 77, 7151–7157 (2011).

Correspondence: Email - vaniaagsantos@hotmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

How Pharma has Responded to the Explosion of New Beta-Lactamases

Karen Bush[1]

[1] Indiana University, Bloomington, IN, USA

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ABSTRACT

Resistance to beta-lactam antibiotics in Gram-negative bacteria is driven most strongly by the presence of beta-lactamases. More than 3,000 unique, naturally-occurring beta-lactamases have been identified, either as enzymes with an active site serine or as enzymes utilizing His-bound Zn atoms to facilitate beta-lactam hydrolysis.

Within the past five years, the US FDA has approved seven new agents that are active against beta-lactamase-producing bacteria, as the result of concerted efforts from the pharmaceutical industry, primarily through small biotech companies. These are listed in the accompanying table. In addition, cefiderocol, a novel siderophore cephalosporin with activity against many beta-lactamase-producing Gram-negative bacteria, is currently under review. The newly approved agents include two tetracyclines and an aminoglycoside, which demonstrate inhibitory activity against carbapenemase-producing bacteria, including many metallo-beta-lactamase-producing pathogens, and beta-lactamase inhibitor combinations including two diazabicyclooctanone (DBO) beta-lactamase inhibitors and a boronic acid beta-lactamase inhibitor that target serine beta-lactamases. Other agents in development include additional DBO and boronic acid inhibitor combinations, all of which are active against pathogens producing serine beta-lactamases, with the potential for clinical activity against metallo-beta-lactamase-producing organisms. Inhibitors specific for bacterial metallo-beta-lactamases may also be on the horizon. Finally, agents stable to hydrolysis by the majority of beta-lactamases of major clinical interest are also in development, including non-beta-lactam-containing molecules. Although there appear to be many options for treatment of beta-lactamase-producing pathogens, financial and commercial support for the development of these agents is waning. Another period of low pharmaceutical engagement threatens to halt the progress that has been recently made in this area.

References:

Bush K. 2018. Past and present perspectives on b-lactamases. Antimicrob Agents Chemother 62:e01076-18.

Drugs@FDA: FDA Approved Drug Products. https://www.accessdata.fda.gov/scripts/cder/daf/ Accessed October 15, 2019.

Page, M. G. P. and K. Bush. 2017. What we may expect from novel antibacterial agents in the pipeline with respect to resistance and pharmacodynamic principles. J. Pharmacokin. Pharmacodynam. doi 10.1007/s10928-017-9506-4.

Pew Charitable Trust. Antibiotics Currently in Global Clinical Development. https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2014/antibiotics-currently-in-clinical-development Accessed October 15, 2019.

Theuretzbacher U, Gottwalt S, Beyer P, Butler M, Czaplewski L, Lienhardt C, Moja L, Paul M, Paulin S, Rex JH, Silver LL, Spigelman M, Thwaites GE, Paccaud J-P, Harbarth S. 2019. Analysis of the clinical antibacterial and antituberculosis pipeline. Lancet Infect Dis 19:e40-50.

Correspondence: Email - karbush@indiana.edu

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Contending with the Black Swans of Resistance

David M. Livermore

Norwich Medical School, University of East Anglia, Norwich, Norfolk, NR4 7TJ

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ABSTRACT

The public of the developed world anticipates that social and advance should and will continue, as during the past century. Further, it expects that governments and international agencies should and will avert and ameliorate hazards and contingencies, from financial crises to climate change. Whether such expectation is realistic (and the notion would have amazed our forefathers) it is self-evidently desirable to avoid future hazard. These points are relevant to antibiotic resistance, which has joined the accepted canon of global threats. There are many calls for better infection control and better antibiotic stewardship, along with suggestions that governments should collectively offer financial incentives to encourage the development of new antibiotics, given that much of the pharmaceutical industry has abandoned this area.

Evidence supports the view that, if strictly enforced, infection control and stewardship can slow the spread of resistance, though it should be added that these good practices are counterpoised to major secular trends driving resistance, including (i) the growth and ageing of populations, (ii) increased travel and migration to and from high resistance areas, as well as (iii) stressedhealthcare systems.

It is, however, simplistic to suppose that the proliferation of resistance solely reflects controllable factors. Rather there are two components. One is the spread of resistance, which is at least partly tractable to infection control and stewardship. But theother is the initial emergence of resistance, often by the random escape of chromosomal genes from harmless environmental organisms to mobile DNA, which is then acquired by pathogens, including strains with epidemic potential. These initial escapes and transfers are unpredictable and uncontrollable 'Black Swan' events. There are many examples but, among the most dramatic and recently consequential are: (i) the escape of CTX-M extended-spectrum -lactamase genes from Kluyvera spp. to opportunistic Enterobacteriaceae and to E. coli ST131 in particular and (ii) the escape with KPC carbapenemases, from an unknown source, to pKpQIL plasmids, acquired by K. pneumoniae ST258. No human intervention can prevent such random events, which seed the emergence of new problems. Nor, crucially, can we predict the nature of the next Black Swan. It may bea carbapenemase that proliferates in E. coli, or it may be a modified PBP giving wide resistance in Enterobacteriaceae just as acquired PBP2' does in MRSA.

These points have great bearing on proposals that governments and agencies should sponsor – e.g. by 'Market Entry Rewards' – the development of new antibiotics to counter emerging and future resistance threats. Unless rewards are spread widely – which is unlikely given the $1 billion sums proposed – this approach is likely to evolve into one of governments picking, or commissioning, a few 'winners' based on the extrapolation of current resistance trends. The hazard is that the agents thereby chosen and rewarded will not be the ones needed to contend with whatever new resistance threats do emerge through unpredictable Black Swan events.

The more resilient route to being able to contend with unknown and unknowable future resistance threats lies in encouraging diversity of development and accepting that this will include failures and redundancies. We simply do not know, nor can we reliably predict, what will be the most useful new antibiotics of the future. Therefore, rather than deluding themselves that they can pick or sponsor a few future winners, governments should reduce development barriers to new antibiotics, as with recent relaxation of trial regulations, encouraging the development of a diversity of approaches. As an example, once -lactamase inhibitors have been successfully trialled with one partner, showing them to be safe and effective, it should be made simple to partner then with alternative -lactams, based on simple safety trials and pharmacodynamic modelling rather than full Phase III studies as are presently demanded. Further, governments should seek ways to support the continued availability of little-used antibiotics with unique activities – experience with both colistin and vancomycin shows how such compounds can suddenly prove useful even after long periods of abandonment.

Correspondence: Email - livermor@claranet.co.uk

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Impact of the universal PCV10 use on carriage with drug-resistant Streptococcus pneumoniae among children in Brazil

Felipe P. G. Neves[1*], Nayara T. Cardoso[1], Tatiana C. A. Pinto[2], Lúcia M. Teixeira[2], Lee W. Riley[3]

[1] Biomedical Institute, Universidade Federal Fluminense, Niterói, RJ, Brazil; [2] Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, RJ, Brazil; [3] School of Public Health, University of California, Berkeley, CA, USA

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ABSTRACT

Brazil was the first country to introduce the 10-valent pneumococcal conjugate vaccine (PCV10) for routine childhood vaccination via Brazil's National Immunization Program in 2010, which is free of charge. Simultaneously, the 13-valent vaccine (PCV13) was made commercially available. In Niterói city, a large metropolitan area in southeastern Brazil, childhood PCV10 vaccination coverage reached 86% among the eligible population (≥ 2 months old) in 2014, in contrast to only 8% of the PCV13, due to its high cost [1]. High PCV coverage in a given population results in a notable decrease in the prevalence of carriage and the incidence of invasive diseases caused by vaccine serotypes. Subsequently, non-vaccine serotypes and non-encapsulated S. pneumoniae associated with both colonization and diseases emerge, a phenomenon termed serotype replacement [2]. Nasopharyngeal carriage is a precondition for pneumococcal transmission and diseases. Here we report the effects after four years of routine PCV use, mostly PCV10, on neumococcal carriage among children aged < 6 years living in Niterói, RJ, Brazil. We analyzed 242 children in 2010 and 573 children in 2014. Compared to data on pneumococcal carriage prior to PCV10 introduction [3], by 2014, the proportion of penicillin non-susceptible pneumococci was similarly high (about 40%). Additionally, non-susceptibility frequencies to erythromycin, clindamycin, and tetracycline became at least 20% higher, mostly because of the emergence of multidrug resistant (MDR) serotype 6C isolates. The direct effect of the PCV13 vaccination is hard to evaluate due to the low coverage of the PCV13 in the population analyzed, but PCV13 serotypes were not found colonizing the children immunized with this vaccine. Increasing PCV13 coverage might help reduce the frequency of major serotypes currently associated with invasive pneumococcal diseases in Brazil, such as 3 and 19A. However, the isolation of MDR serotype 6C and non-typeable isolates in carriage among children immunized with either PCV10 or PCV13 requires close monitoring. Antimicrobial resistance in S. pneumoniae has been continuously increasing, mostly due to the global spread of MDR clones, such as those recognized by the Pneumococcal Molecular Epidemiology Network (PMEN) [4]. We observed that about 35% of the children were colonized with 15 different PMEN clones or closely related lineages before (2010) and after (2014) PCV implementation. Such lineages were mainly responsible for high antimicrobial resistance frequencies. Several clones presented penicillin nonsusceptibility in the pre- and post-PCV10 periods, but the majority of the isolates belonged to a few clones, predominantly serotype 14-ST156 and serotype 6C-CC386, respectively. In turn, erythromycin resistance was polyclonal only after four years of PCV10 routine vaccination, but high resistance frequencies were largely explained by MDR serotype 6C-CC386, a lineage genetically related to the Poland6B-20 clone. We also observed capsular switching events involving serogroups 6 and 23 clones, which may be a pneumococcal escape mechanism [5]. Ongoing surveillance of pneumococcal clonal composition is important to evaluate PCV use outcomes and to identify factors other than PCV that drive pneumococcal drug-resistance evolution.

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References:

[1] F.P.G. Neves, N.T. Cardoso, R.E. Snyder, M.A. Marlow, C.A.A. Cardoso, L.M. Teixeira, L.W. Riley. Vaccine 35 (2017) 2794–2800.

[2] D.M. Weinberger, R. Malley, M. Lipsitch. The Lancet 378 (2011) 1962–1973.

[3] F.P.G. Neves, T.C.A. Pinto, M.A. Corrêa, R.A. Barreto, L.S.G. Moreira, H.G. Rodrigues, C.A. Cardoso, R.R. Barros, L.M. Teixeira. BMC Infect Dis 13 (2013) 318.

[4] PMEN: Pneumococcal Molecular Epidemiology Network [Internet]. [cited 2017 May 4]. Available from: http://www.pneumogen.net/pmen/

[5] F.P.G. Neves, N.T. Cardoso, A.R.V. Souza, R.E. Snyder, M. Marlow, T.C.A. Pinto, L.M. Teixeira, L.W. Riley. J Antimicrob Chemother 73 (2018) 1206–1212.

Correspondence: Email - fpgneves@id.uff.br

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Influence of *Lactobacillus reuteri* on the regulation of inflammasome genes expression during campylobacteriosis in broiler

V. Karaffová[1]*,V. Revajová[1], M. Šefcová[1], S. Gancarčíková[2], Z. Ševčíková[1], M. Sr Levkut[1,3]

[1] Insitute of Pathological Anatomy; [2] Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovakia; [3] Neuroimmunological Institute SAS, Dúbravská cesta 9, 845 10 Bratislava, Slovakia

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ABSTRACT

Campylobacteriosis was the most commonly reported zoonosis and the increasing European Union (EU) trend for confirmed human cases since 2008 stabilised during 2012–2016. In food, the occurrence of Campylobacter remained high in broiler meat [1]. Moreover, broiler immunesystem is inefficiently activated against C. jejuni colonization and the expression of keys antimicrobial peptide genes is suppressed [2]. Inflammasomes are multiprotein complexes that form in the cytosol following sensing of intracellular threats like intruding bacteria and viruses or cell damage. After assembly, inflammasome induce the activation of caspase-1, which subsequently activates cytokines IL-1β and IL-18, and induce a form of cell death called as pyroptosis. Furthermore, disregulation of inflammasomes may result in impaired host-defense against bacterial pathogens [3]. In a recent study has shown that probiotic bacteria can modulate key biological signalling pathways of inflammation [4].The aim of this study was to evaluate the influence of Lactobacillus reuteri B1/1 on the regulation of inflammasome in caecum of broilers challenged with Campylobacter jejuni CCM6189. Seventy two one day-old chicks were randomly divided into 4 experimental groups (n= 24): control (C), *L. reuteri* (LB), *C. jejuni* (CJ), and combined *L. reuteri* + *C. jejuni* (LBCJ). *L. reuteri* at the concentration of 109 CFU/0.2 ml in Ringer's' solution was administered daily per os to selected groups from day 1 to day 7 ofthe experiment. *C. jejuni* was administered orally on day 4 of the experiment by a single dose of 1x108 CFU/0.2 ml PBS to selected groups. Samples from the caudal part of the caecum for isolation of mRNA of target genes were collected 12, 24 and 48 hours after infection by C. jejuni (dpi). Samples were homogenized and total of RNA was isolated. Amplification and detection of specific products were performed using CFX 96 RT system (Bio-Rad, USA) with predefined program. Relative mRNA expression of target genes (Casp-1, IL-1β, IL-18) was mainly upregulated in combined group compared to other groups ($P < 0.05$; $P < 0.01$; $P<0.001$) 24 hours after infection. The administration of *L. reuteri* strongly modulated inflammasome genes expression in caecum, thereby increasing the capacity of the immune system to react to the presence of *C. jejuni* in the early phase of infection in chickens.

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References:

[1] EFSA - scientific report, EFSA Journal 15 (2017) 5077.
[2] L. Bouwman, M.R. de Zoete, N.M. Bleumink-Pluym, R.A. Flavell, J.P. van Putten, Inflammasome activation by Campylobacter jejuni, J Immunol 193 (2014) 4548–4557.
[3] T. Strowig, J. Henao-Mejia, E. Elinav, R. Flavell, Inflammasomes in health and disease, Nature 481 (2012) 278–286.
[4] G.S. Howarth, H. Wang, Role of endogenous microbiota, probiotics and their biological products in human health, Nutrients5 (2013) 58 –81.

Correspondence: Email - viera.karaffova@uvlf.sk

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

The mega-plasmid reported worldwide confers multiple antimicrobial resistance in *Salmonella Infantis* of broiler origin in Russia

A. Bogomazova[1]*, V. Gordeeva[1], E. Davydova[1], E. Krylova[1], A. Sukhoedova[1], I. Soltynskaya[1], A. Komarov[1]

[1] The Russian State Center for Animal Feed and Drug Standardization and Quality (FGBU«VGNKI»), Moscow, Russia

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ABSTRACT

Plasmids, which refer to a mobile part of the bacterial genome, can acquire and carry over genes of ntimicrobial resistance.Thereby plasmids contribute to the rapid adaptation of the bacterial community to the human-defined environment. In 2014, Israeli scientists reported a large conjugative mega-plasmid pESI (plasmid for emerging S. Infantis) that provides multiple drug resistance (MDR) of Salmonella Infantis isolated from broilers [1]. Later, the very similar pESI-like plasmids were reported in Salmonella isolated from poultry in the United States, Italy, Switzerland, Hungary, and Japan [2, 3, 4]. Here we report the detection of the pESI-like plasmid in Salmonella Infantis isolated from food chicken products in Russia. Whole-genome sequencing of three MDR isolates revealed pESI-like plasmid in all three cases. This plasmid had such typical pESI features as an operon for siderophore yersiniabactin, a cluster of IncI conjugative genes, a type IV pilusgene cluster, three toxin-antitoxin modules, and class 2 integron. The pESI-like plasmid carried from 2 to 5 resistance genes in each isolate. In total, we observed 6 antimicrobial resistance (AMR) genes associated with pESI-like plasmids (aadA1, blaCTX-M-14, dfrA14, sul1, tetA/tetR, tetM). Besides plasmid genes of antimicrobial resistance, all three MDR isolates of Salmonella Infantis harbored mutations in the chromosomal gyrA gene (p.S83Y or p.D87Y) and parC gene (p.T57S), which are associated with resistance to fluoroquinolones. Also, we performed a bioinformatic meta-analysis. This analysis showed the presence of pESI-like plasmids in Salmonella Infantis not only from the US, Europe, Israel, and Japan but from Chile and Peru. Thus, one can suspect the worldwide spread of pESI-like plasmid among Salmonella Infantis linked with chicken poultry.

isolate / drug class	S-11	S-12	S-13
beta-lactam			**blaCTX-M-14***
fluoroquinolones	gyrA.p.D87Y, parC.p.T57S	gyrA.p.S83Y, parC.p.T57S	gyrA.p.S83Y, parC.p.T57S
tetracyclines	**tetA*/tetR***	**tetA*/tetR***	**tetA*/tetR***, two copies of **tetM***
aminoglycosides	**aadA1***, AAC(6')-Iaa	AAC(6')-Iaa	AAC(6')-Iaa
sulfonamides	**sul1***		
trimethoprim	**dfrA14***	**dfrA14***	**dfrA14***

* – bold font indicates plasmid genes.
Table 1. AMR genes revealed by ResFinder tool.

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References:

[1] Aviv, G., Tsyba, K., Steck, N., Salmon-Divon, M., Cornelius, A., Rahav, G., Gal-Mor, O. (2014). Environmental microbiology, 16(4), 977-994.
[2] Franco, A., Leekitcharoenphon, P., Feltrin, F., Alba, P., Cordaro, G., Iurescia, M. Hendriksen, R.S. (2015). PLoS One, 10(12), e0144802.
[3] Tate, H., Folster, J. P., Hsu, C. H., Chen, J., Hoffmann, M., Li, C., Green, A. (2017). Antimicrobial agents and chemotherapy, AAC-00488.
[4] Szmolka, A., Szabó, M., Kiss, J., Pászti, J., Adrián, E., Olasz, F., & Nagy, B. (2018). Food microbiology, 71, 25-31.

Correspondence: Email - sequence@vgnki.ru

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

WGS characterization of multidrug-resistant *Enterococci* isolated from reindeer in the Russia

E. Krylova*, A. Bogomazova, V. Gordeeva, E. Davydova, A. Sukhoedov, I. Soltynskaya, A. Komarov

The Russian State Center for Animal Feed and Drug Standardization and Quality (FGBU«VGNKI»), Moscow, Russia

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ABSTRACT

The monitoring for antimicrobial resistance (AMR) among zoonotic bacteria is an essential part of surveillance for the safety of food chain. Thus, the national AMR program in Russia includes a monitoring performed by veterinary specialists. The Enterococcus spp. is among bacteria included in such a monitoring. The Enterococcus spp. is a common bacteria found in multiple animal hosts and products of animal origin. Earlier this bacteria was considered a harmless commensal residing in the intestine. Recently it has emerged as a multiple-drug-resistant virulent pathogen accounting for nosocomial infections. Here we report the results of a search for AMR genes in five multi-drug resistant (MDR). Enterococci isolates was obtained from animal farms (reindeer, cows) near Naryan-Mar beyond the Arctic Circle. The isolates were tested resistance antimicrobial used in veterinary. Bacterial genomic DNA was extracted by silica gel adsorption. Library preparation was performed using Illumina Nextera® XT. Illumina paired-end genome sequencing 2x300 bp was performed using Miseq platform. The WGS reads were de novo assembled using SPAdes. The annotation of assembled contigs was performed on the RAST annotation server. The ResFinder, ARGANNOT and CARD tools were used to identify resistance determinants. Each isolat harbored from 4 to 10

isolate / drug class	M-1	M-2	M-13	M-16	M-18
origin	cattle	cattle	reindeer	reindeer	reindeer
MLST	ST-25, E.faecalis	ST-1046, E. faecium	ST-133, E.faecalis	ST-133, E.faecalis	ST-133, E.faecalis
fluoroquinolones		efmA			
tetracyclines	tetL, tetM		tetS		tetS
phenicols	**catA7***		**catA9***		**catA9***
macrolides	**ermB***, lsa(A)	efmA, msrC, lsa(A)	lsa(A)	lsa(A)	lsa(A)
streptogramines	**ermB***	msrC			
aminoglycosides	**APH(3')-IIIa*, ANT(6)-Ia***	AAC(6')-Ia	**APH(3')-IIIa*, ANT(6)-Ia***		**APH(3')-IIIa* ANT(6)-Ia***
glycopeptides			vanS, vanR	vanS, vanR	vanS, vanR
trimethoprim	**dfrG***, dfrE		dfrE	dfrE	dfrE

* – bold font indicates plasmid genes.

resistance genes conferring resistance to phenicols, macrolides, streptogramines, aminoglycosides, tetracyclines, glycopeptides, fluoroquinolones and trimethoprim.

Each isolate contained up to 3 plasmids. The one plasmid was similar to pRE25 described earlier for Enterococcus isolates (animal, food, human) from European countries [1]. The revealing of Enterococcus conjugative MDR plasmid similar to ones discovered earlier is an excellent example of how easily a determinant of resistance can spread. Prudent uses of antimicrobials in human and animal medicine are necessary, as well as the implementation of international measures to control zoonotic pathogens and limit the global emergence of resistance.

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References:

[1] Rosvoll, Torill CS, et al FEMS Immunology & Medical Microbiology 58.2 (2010): 254-268.

Correspondence: Email - sequence@vgnki.ru

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Knowledge and attitudes on antibiotic use and antimicrobial resistance among veterinary and agriculture students

Zorana Kovacevic[1], Bojan Blagojevic1

[1] Department of Veterinary Medicine, University of Novi Sad, Novi Sad, Serbia

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ABSTRACT

Antimicrobial resistance (AMR) represents one of the biggest threats to global health today and it is connected with the lack of knowledge among general population. Irrational use of antibiotics is directly associated with AMR and Serbia belongs to a group of European countries with the highest rates of AMR.This study aimed to evaluate present status of the knowledge and attitudes of the veterinary (VS) and agriculture students (AS) towards antibiotic use and AMR in order to assess the impact of the medical education on students' knowledge and attitudes towards antibiotics. The study was conducted at the Faculty of Agriculture, University of Novi Sad, among 105 veterinary students (3rd, 4th and 5th year of thestudies) and 99 students of agricultural sciences (last year of the studies). All approached students agreed to complete anonymous questionnaire. In contrast to AS, VS attended veterinary pharmacology course. The average age of students was 22.7 years and 43.9% were females. Self-medication with antibiotics was admitted by 48.5% of the total sample and 25.9% of the respondents used antibiotics until their symptoms disappeared regardless of the period of prescription. In Groups VS and AS, 34.3% and 62.6%, respectively, of the respondents believed that antibiotics could be used to cure common cdd. Around a third of the respondents in both groups thought that treatment with antibiotics should be started on the basis of pharmacist's advice. Around 4.8% of the students (Group VS) and 9.1% (Group AS) said that they started their treatment with antibiotics that were already stocked at home. Roughly 97.1% (Group VS) and 93.9% (Group AS) of respondents claimed that the antibiotic treatment should be started after a visit to a medical doctor and by receiving a prescription. This study has indicated that VS showed better knowledge on AMR compared to AS students which was expected as they attended veterinary pharmacology course. Nevertheless, there are still some areas of misconceptions regarding antibiotic useand AMR, even in the VS group. Therefore, further interventions should be focused on educational campaigns targeting the behavior of university students with regard to antibiotic use and improvement of their perceptions on AMR.

Table 1: Knowledge on the antibiotic use among veterinary (VS) and agriculture students (AS). T/F: true/false and percentages denote those who said "True".

Questions	True/Falsen (VS)	% True (VS)	True/Falsen (AS)	% True (AS)
A. Reason to use antibiotic:				
To decrease pain (T/F)	12/93	11.4	45/54	45.4
To decrease fever (T/F)	26/79	24.8	46/53	46.5
To overcome malaise and fatigue (T/F)	3/102	2.9	11/87	11.1
To cure common cold (T/F)	36/69	34.3	62/37	62.6
B. Antibiotics therapy could be started:				
With an antibiotic found at home in order not to waste time (T/F)	5/100	4.8	9/90	9.1
With prescription (T/F)	102/3	97.1	93/6	93.9
After recommended by a pharmacist (T/F)	34/71	32.4	39/60	39.4

References:

[1] World Health Organization. Antibiotic resistance: Multi-country public awareness survey. 2015. Available from: http://www.who.int/drugresistance/documents/baselinesurveynov2015/en/

[2] TNS Opinion. Special Eurobarometer 445: Antimicrobial Resistance. https://doi.org/10.2875/760366 Available from: https://ec.europa.eu/health/amr/sites/amr/files/eb445_amr_generalreport_en.pdf.

[3] O.J. Horvat, A.D. Tomas, M.M.P. Kusturica, A.V. Savkov, D.U. Bukumirić, Z.S. Tomić, et al; PLoS ONE. (2017) 12(7): e0180799.

[4] Y. Huang, J. Gu, M. Zhang, Z. Ren, W. Yang, Y. Chen&F. Zhang; BMC medical education, (2013) 13(1), 163.

[5] D. Limaye, S. Naware, P. Bare, S. Dalvi, K. Dhurve, A. Sydymanov, V. Limaye, R.S. Pitani, Z. Kanso, G. Fortwengel. Int. J. Res. Med. Sci., (2018) 6,1908-1912.

Correspondence: Email - zorana.kovacevic@polj.edu.rs

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Heavy metal and antibiotic resistance genes in bacteria from porcine monophasic *Salmonella*

Helen Billman-Jacobe[1,2*], Mike Dyall-Smith[1], Jane Hawkey[2] and Yuhong Liu[1]

[1] Asia-Pacific Centre for Animal Health, Department of Veterinary Biosciences, Melbourne Veterinary School, University of Melbourne; [2] National Centre for Antimicrobial Stewardship, Peter Doherty Institute, Melbourne, Australia.

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EXTENDED-ABSTRACT

Introduction:

Historically, Australian pigs have had low estimated Salmomella Typhimurium prevalence relative to European herds. However, recent data suggest that the prevalence of a monophasic variant, Salmonella enterica serotype 1,4,[5],12:i:- has increased to the point where it may have surpassed S. Typhimurium in primary production [1].The main objective of this study was to understand the genetic relationships of S. 1,4,[5],12:i:- isolates recovered from pig faeces from multiple, widely dispersed, commercial pig farms. A previous study using MLVA indicated that fairly stable populations of S. 1,4,[5],12:i:- were circulating within pig herds [1]. MLVA uses a small panel of repetitive loci to compare isolates and has largely been replaced by whole genome sequence analysis (WGS). In this study, the WGS of the isolates were determined and the phylogenetic relationships were inferred. The chromosomally-encoded antibiotic resistance genes (ARGs) and heavy metal resistance genes (HMRGs) were compared.

Materials and Methods:

Salmonella were isolated from fresh pen-floor pig faecal samples from 6 farms and an abattoir, as previously reported [1].Genomic DNA from pure isolates was extracted using the JANUS Chemagic automated workstation (PerkinElmer®) with the Chemagic Viral DNA/RNA kit (PerkinElmer®). Unique dual indexed libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina®). Libraries were sequenced on the Illumina NextSeq® 500 with 150-cycle paired end chemistry as described by the manufacturer's protocols.
For phylogenetic analysis, the short read libraries of 55 isolates sequenced in this study and 31 isolates obtained from the European Nucleotide Archive (ENA) were used for phylogenetic and CRISPR analysis. All isolates were mapped to the reference genome S. 1,4,[5],12:i:- strain TW-Stm6 (Genbank accession CP019649,[2] using RedDog v1beta10.3 (https://github.com/katholt/RedDog) with default parameters. Briefly, Illumina reads were mapped using Bowtie2 v2.2.9 using the sensitive local algorithm with a maximum insert length of 2000 (set with the x parameter) [3]. SNPs were called using SAMtools v1.3.1, and alleles at each locus were determined by comparing to the consensus base in that genome, using SAMtools pileup to remove low quality alleles (base quality <= 20, read depth <=5 or a heterozygous base call)[4]. SNPs found in repeat regions (including phage, tandem repeats and horizontally transferred regions) were removed. Gubbins v2.1.0 was used to detect SNPs in recombinant regions and these SNPs were excluded [5]. Amongst the 86 isolates, 386 SNPs were detected (226 non-synonymous SNPs, 85 synonymous SNPs and 76 intergenic SNPs).This final SNP alignment was used to construct a tree in RAxML v8.2.8 using a GTR+G substitution model with 100 bootstraps. Five independent RAxML trees were generated, and the tree with the best likelihood was selected for downstream analysis [6].
CRISPR analysis was performed using pair-end Illumina reads of each isolate which were assembled using SPAdes (3.11.0) with the "careful" option to reduce the number of mismatches and shorts indels [6]. The CRISPR Recognition Tool was used to identify direct repeats and spacers [7]. The acceptable length of repeat and spacer regions were 19-38 bp and 19-48 bp, respectively. CRISPR Finder was used to set the orientation of spacer arrays for each isolate [8]. Spacers started with the one in the distal end of leader to the one close to leader. CRISPR spacer sequences were aligned in a table and visualized using R (http://www.R-project.org/).

Correspondence: Email - hbj@unimelb.edu.au

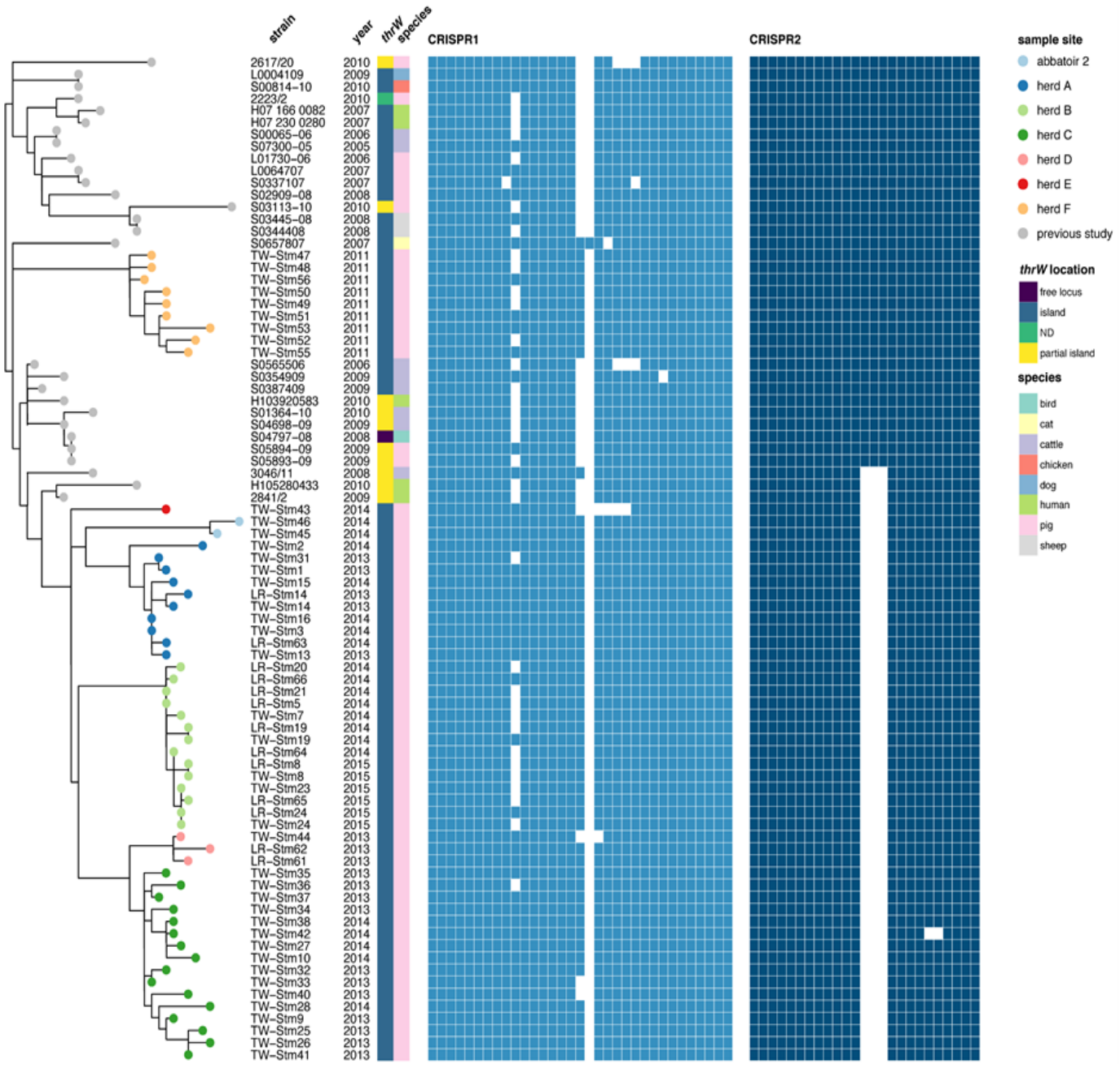

Figure 1: Phylogenetic tree and CRISPR array comparison of Australian porcine S. 1,4,[5],12:i:- isolates (colored tips) and European S. 1,4,[5],12:i:- isolates (grey tips) from various sources.

Results:

Longitudinal sampling from each farm yielded isolates that were highly related as shown by the tight clustering of isolates from each source (herds A-F, Abattoir) (Figure 1). Furthermore, there was little diversity in the core genome of isolates between farms (≤81 SNPs) and isolates from the same source clustered together indicating each farm had a dominant, persistent clone (≤20 SNPs). Figure1 also shows the close relationship of the Australian isolates with S. 1,4,[5],12:i:- isolates belonging to the current European epidemic clade [9].

Comparison of the thrW genomic island [9] and the CRISPR arrays [10] did not distinguish isolates according to their geographic origin. The S.1,4,[5],12:i:- chromosomes carry heavy metal resistance genes on a genomic island, SGI-4 which was conserved in all of the isolates from this study [11]. Some isolates from herd B also carried a large, conjugative plasmid conferring antibiotic resistance and copper and silver tolerance [12]. The plasmid-encoded metal tolerance genes (pco-sil) were associated with a Tn7-like mobile element (Fig. 2A, filled-black arrows). Typically, members of the European S. 1,4,[5],12:i:- clade have a resistance locus containing multiple antibiotic resistance genes and the mer operon (Figure 2B). This locus was

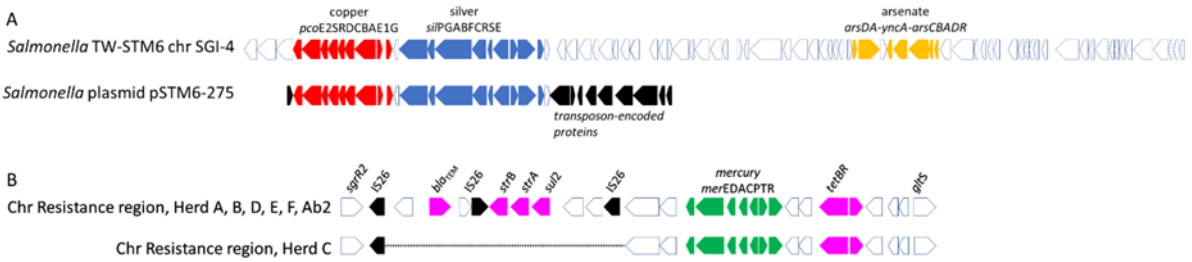

Figure 2: Comparison of the arrangement of heavy metal resistance genes of SGI-4 and pSTM6-275 (A). Comparison of the chromosomal resistance regions of isolates from different herds (B).

detected in most of the Australian isolates with the exception of isolates from herd C where the blaTEM, strAB and sul2 genes were absent, presumably via an IS26-mediated deletion.

Conclusions:

Salmonella 4, [5],12:i- was isolated from the faeces of healthy pigs from multiple pig farms. All isolates from each farm were highly similar to each other suggesting that biosecurity measures have been effective in preventing multiple introductions of Salmonella. The similarity of WGS of isolates from different farms suggest that they share a recent common ancestor with the European epidemic clade [9]. The loss of chromosomal antibiotic resistance genes (blaTEM, strAB and sul2), for example in herd C isolates, suggest that these isolates were not under strong selection by amoxycillin, streptomycin and/or sulphonamides.

Acknowledgments:

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References:

[1] T. Weaver, M. Valcanis, K. Mercoulia, M. Sait, J. Tuke, A. Kiermeier, G. Hogg, A. Pointon, D. Hamilton, H. Billman-Jacobe, Prev Vet Med, 136 (2017) 19-28. 10.1016/j.prevetmed.2016.11.010: 10.1016/j.prevetmed.2016.11.010

[2] M.L. Dyall-Smith, Y. Liu, H. Billman-Jacobe, Genome Announc, 5 (2017). 10.1128/genomeA.00793-17: 10.1128/genomeA.00793-17

[3] B. Langmead, S.L. Salzberg, Nature Methods, 9 (2012) 357.

[4] H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, Bioinformatics (Oxford,England), 25 (2009) 2078-2079. 10.1093/bioinformatics/btp352: 10.1093/bioinformatics/btp352

[5] N.J. Croucher, A.J. Page, T.R. Connor, A.J. Delaney, J.A. Keane, S.D. Bentley, J. Parkhill, S.R. Harris, Nucleic acids research, 43 (2015) e15. 10.1093/nar/gku1196: 10.1093/nar/gku1196

[6] A. Stamatakis, Bioinformatics (Oxford, England), 30 (2014) 1312-1313. 10.1093/bioinformatics/btu033: 10.1093/bioinformatics/btu033

[7] C. Bland, T.L. Ramsey, F. Sabree, M. Lowe, K. Brown, N.C. Kyrpides, P. Hugenholtz, BMC bioinformatics, 8 (2007) 209. 10.1186/1471-2105-8-209: 10.1186/1471-2105-8-209

[8] I. Grissa, G. Vergnaud, C. Pourcel, Nucleic acids research, 35 (2007) W52-57. 10.1093/nar/gkm360: 10.1093/nar/gkm360

[9] L. Petrovska, A.E. Mather, M. AbuOun, P. Branchu, S.R. Harris, T. Connor, K.L. Hopkins, A. Underwood, A.A. Lettini, A. Page, M. Bagnall, J. Wain, J. Parkhill, G. Dougan, R. Davies, R.A. Kingsley, Emerg Infect Dis, 22 (2016) 617-624. 10.3201/eid2204.150531: 10.3201/eid2204.150531

[10] L. Fabre, J. Zhang, G. Guigon, S. Le Hello, V. Guibert, M. Accou-Demartin, S. de Romans, C. Lim, C. Roux, V. Passet, PloS one, 7 (2012) e36995.

[11] P. Branchu, O.J. Charity, M. Bawn, G. Thilliez, T.J. Dallman, L. Petrovska, R.A. Kingsley, Front Microbiol, 10 (2019) 1118. 10.3389/fmicb.2019.01118: 10.3389/fmicb.2019.01118

[12] H. Billman-Jacobe, Y. Liu, R. Haites, T. Weaver, L. Robinson, M. Marenda, M. Dyall-Smith, Antimicrob Agents Chemother, 62 (2018). 10.1128/AAC.02357-17: 10.1128/AAC.02357-17

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE 2019 (IC2AR 2019)

Principles of the antimicrobial control system organization in veterinary medicine and agriculture of the Russian Federation

Svetlana Shchepetkina[1]

[1] Saint-Petersburg State Academy of Veterinary Medicine, Russian Federation.

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Extended-Abstract

Russian Federation has ensured the country's food security, adopted a policy of increasing the export potential, which entails an increase in agricultural production. According to the Ministry of Agriculture of the Russian Federation, the volume of exports of agricultural products and food in 2018 amounted to $ 25.7 billion, which is 19% more than in 2017. The main growth was due to an increase in grain exports to $ 10.5 billion - almost 40% more than in 2017. The percentage of fish and seafood was 19.9 %, food&processing industry and oil&fat products-13.6 % and 12.3 %.

Scaling production entails scaling problems. The prerequisites for the current situation in livestock breeding arose in the 2000s, when the main task was to ensure the country's food security.

Production was growing steadily, but most enterprises tried (and are trying!) to get more products from one square meter of the area by compacting the planting. An additional risk factor is antibiotics to stimulate the growth and productivity of animals and birds, as well as the uncontrolled use of antimicrobial drugs for the treatment and prevention of bacterial diseases. Thus, in huge quantity of Russian poultry farms use from 2 to 4 courses of antibiotic therapy per broiler chicken growing cycle (40-44 days) [2].

A compacted conditions, a constant immune load entails a decrease in the resistance of the organisms an increase in the number of manifestations of infectious diseases of bacterial, parasitic, viral etiology.

This led to accelerated reproduction of microbes, passage and increased virulence, as a result - there was an additional immune load and opportunistic infections appeared, caused by the body's own microflora.

The result is clearly visible on the example of salmonellosis - one of the main problems of modern poultry farms. Salmonella normally lives in the blind processes of the intestines of the bird, but usually does not cause disease. However, under stress (tightening of the planting, improper vaccination schedule, etc.), it multiplies intensely, enters the intestinal lumen and then the environment. The fight against infection leads to an increase in the use of antibiotics, as a result of which are resistant to them Salmonella and the presence of antimicrobial agents in the finished product and the human body. These factors stimulate the development of antibiotic resistance - a global problem of the 21st century.

In June of this year, AMPs of the fluoroquinolone group were found even in the eggs of hens from a number of leading Russian poultry farms. Fluoroquinolones are prohibited for use by children under 18 years because of the risk of arthropathy, and in the elderly people they provoke a convulsive syndrome.

The Russian controlling organizations Rosselkhoznadzor, Rospotrebnadzor, Roskontrol reveal the antibiotics in food everywhere. For example, during 2017, excess residues of antibiotics of the tetracycline group in finished products were repeatedly detected by 15-17-21 times. At the same time, tetracycline is prohibited for use by pregnant women and children under 8 years of age, as it causes irreversible changes in the bones of the longitudinal skeleton and long-term darkening of the tooth enamel in children.

We found that a decrease in sensitivity to the antibiotic occurs already after the first course of use. When a new antibiotic is introduced into the Russian market, its sensitivity to it decreases for one year. So, for 2013-2015, the sensitivity of microorganisms decreased significantly: to fluoroquinolones - by 27.0%, to tetracyclines - from 52.1 to 67.3%, to aminoglycosides - from 11.2% to 41.8% (data range indicated within the pharmacological group) [3].

There is only one way to cope with the problem of antibiotic resistance - painstaking and systematic organization of a system of preventive, diagnostic, anti-epizootic, veterinary-sanitary and general business activities. A competent team and specialists are needed: veterinarians, livestock specialists, agronomists,etc. Each specialist is rooting for his own area of work and for the

Correspondence: Email - vetsvet77@yandex.ru

overall result. But the role of the veterinarian should be the main one, because diseases lead to losses or to the production of dangerous products.

For the 2017-2018, we have developed principles for monitoring pathogens of bacterial etiology and susceptibility of microorganisms to the antibiotics for veterinary use at critical points of the technological cycle of enterprises for growing poultry, pigs and milk production.

A methodology has been developed to reduce the number of used antibiotics in industrial animal husbandry by optimizing the prophylactic epizootic (immunity monitoring, vaccination), veterinary and sanitary (disinfection, compliance with sanitary gaps), general economic (technology, stress prevention, alternative drugs) measures; control of the presence of microorganisms, their sensitivity [1] and withdrawal period after the application in each batch of products (the batch is taken by the population treated with antibiotics according to a single scheme) [2.3].

The work was carried out in 2017-2018 in the Belgorod region - the largest region in meat production in the Russian Federation. In 2018, 1 million 698 thousand tons of meat was produced in live weight, of which 860 thousand tons of pork and 705 thousand tons of poultry meat.

In 2018, an enterprise producing more than 90 types of poultry meat received a permit for labeling products "Antibiotic free". In 2018, it sold 17% of the total regional production. It took two years to reduce the use of antibiotics to almost zero, in the process a complete rejection of coccidiostatics.

Next one is the largest producer and exporter (more than 50% of full export in RF) of eggs in Leningrad region. The work for producing “antibiotic free eggs” took about three years (from autumn 2016). Today at one time are kept more than 5,500,000 heads of laying hens. The duration of the productive period is 630 days. Productivity indicators are higher than the level of genetic characteristics declared for the cross. From 2016 to 2019, egg production increased by 18.1%. The export of products is about 31.75 million eggs per month. The poultry farm provides more than 50% of egg exports to the Russian Federation.

At present, work on the organization of an antimicrobial control system (ASC) is being carried out at other farmers of meat, eggs, milk production in the Russian Federation.

References:

[1] Shchepetkina, S.V. Susceptibility disks for determining sensitivity to antimicrobial drugs used in veterinary medicine // Catalog of innovative developments of agricultural universities // Ministry of Agriculture of Russia, p. 262

[2] Shchepetkina, S.V. Collective Monograthy, SPb., Publishing house FSBUU VPO "SPbGAVM", 536 p.

[3] Shchepetkina, S.V., Novikova O.B., Zabrovskaya, A.V., Terletsky, V.P., Tyshchenko, V.I., SPb .: Publishing house FSBUU VPO "SPbGAVM", 2015. - 160 p.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Two thousand 19: Outcomes of a decade of antimicrobial resistance proteomics

Gilberto Igrejas[1-3*], Luís Pinto[1-4], Patrícia Poeta[3,4]

[1] Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [2] Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal; [3] LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Lisbon, Portugal; [4] Veterinary Science Department, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal.

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ABSTRACT

Antimicrobial resistance (AMR) is today acknowledged to be one of the most concerning threats to global human health and the international community has now started to act concertedly to tackle this problem. AMR is not a recent problem dating back to the discovery and first use of antibiotics. For the last 10 years, the Functional Genomics and Proteomics Unit based at the University of Trás-os-Montes and Alto Douro, Vila Real, Portugal, has aimed to understand more about the mechanisms that equip bacteria to survive antibiotic action by investigating the entire complement of proteins expressed by resistant strains. The proteome can reveal the complex profiles of expressed proteins underlying phenotypic characteristics, and provide an excellent approximation of the information contained within a microorganism's genome. Coupled with bioinformatics, proteomics has led to significant progress in the characterization of bacterial pathogens. From different starting points, the proteomics studies performed have contributed to knowledge on over 2000 proteins that individually or as a complex whole participate in AMR. Since 2009, the proteomes of different *Salmonella, Enterococcus, Escherichia coli* and *Staphylococcus aureus* strains have been thoroughly studied with the purpose of identifying the main proteins present or those that are differentially expressed between strains. By looking at the whole proteome or subfractions thereof, resistant strains with different levels of resistance have been compared to related non-resistant strains both in the presence and absence of antibiotic stress. High-resolution protein separation has been achieved with two-dimensional gel electrophoresis (2-DE) and shotgun analysis has overcome some of the limitations related to the low solubility of membrane proteins. Comprehensive coverage of the proteins present has thus been attained by using 2-DE followed by either matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) together with shotgun LC-MS/MS approaches. The study of strains recovered from clinical human samples provided a better understanding of extended-spectrum beta-lactamase (ESBL) producing *E. coli*, new insights into pleural empyema methicillin-resistant *S. aureus*, and an in-depth examination of the mechanisms of quinolone resistance in *Salmonella Typhimurium* causing acute gastroenteritis. Other proteomes studied were those of multidrug resistant (MDR) *E. coli* and ciprofloxacin resistant and vancomycin resistant enterococci recovered from pigs slaughtered for human consumption. Also, the role of resistant bacteria as environmental reservoirs of AMR was better elucidated through studying the proteomes of ESBL-positive *E. coli*, vanA-positive enterococci and MDR *Salmonella* recovered from a variety of free-ranging wild animals including boars, rabbits, seagulls, red foxes, Iberian wolves and lynxes. Proteomics techniques have considerably improved during the past decade in parallel with advances in bioinformatics, and together have proved to be the appropriate research tool with which to tackle the major challenge that AMR poses in modern medicine.

Acknowledgments:

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Correspondence: Email - gigrejas@utad.pt

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Comparative membrane-associated proteome of *Leishmania* (*Leishmania*) *infantum* and *L.* (*L.*) *amazonensis*

Hélida M. de Andrade[1]*, Ivana H.R. Oliveira[1], Henrique C. P. Figueiredo[2], Cristiana P. Rezende[2], Thiago Verano-Braga[3], Marcella N. Melo-Braga[4].

[1] Departamento de Parasitologia, ICB/UFMG, Brazil; [2] Aquacen, UFMG Brazil; [3] Departamento de Fisiologia e Biofísica, ICB/UFMG Brazil; [4] Departamento de Bioquímica e Imunologia, ICB/UFMG Brazil

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ABSTRACT

The species *L. (L.) infantum* and *L. (L.) amazonensis* are causative agents of visceral and cutaneous leishmaniasis, respectively. Most proteome analyses of Leishmania have been carried out on whole-cell extracts, but this approach tends to underrepresent membrane proteins because of their high hydrophobicity and low solubility. Due to the great importance of membrane proteins in biological processes, including host–parasite interactions, virulence and invasiveness, this study applied label-free shotgun proteomics to characterize and evaluate abundance levels of plasma membrane proteins of promastigotes life-stage. The total number of proteins identified in *L. (L.) infantum* and *L. (L.) amazonensis* was 2033 and 2243, respectively, and both species shared 1908 of these proteins quantified. After cell localization prediction of all identified proteins, 394 were described as plasma membrane proteins and their majority (320 proteins) was shared between both species, 18 were exclusively detected in *L. (L.) infantum* and 56 in *L. (L.) amazonensis*. Proteins with qualitative (present or absent) or quantitative (p-value < 0.05) differences were classified as "regulated" proteins. Thus 106 regulated plasma membrane proteins were selected, being 32 from *L. infantum* and 74 from *L. amazonensis*. These proteins were representing considerably distinct processes in the species, such as regulation of cell communication and cell adhesion in *L. (L.) infantum*, and localization and transport metabolism in *L. (L.) amazonensis*. The proteins involved in these processes included GP63, a well-known virulence factor, as well as members of the ABC transporter superfamily. The identification of numerous proteins with uncharacterized roles highlights the importance of investigating Leishmania proteins. They could contribute to better understand the parasite biology and may act as new potential therapeutic targets.

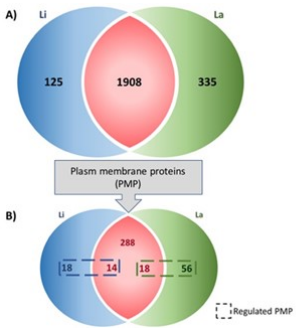

Figure 1 - Distribution of identified proteins. *L. (L.) infantum (L.i)* and *L. (L.) amazonensis (L.a)* exclusive and shared proteins before and after plasm membrane protein (PMP) selection and PMP up regulated in each species.

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Correspondence: Email - helidandrade@gmail.com

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In pathogen bacteria *Staphylococcus aureus* MazEF Toxin-Antitoxin System (TAS) regulates cell dormancy in response to environmental stress

Olesya O. Panasenko[1,2*], Fedor Bezrukov[3], Julien Prados[1], Roberto Sierra[1], Adriana Renzoni[1,2]

[1] Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211 Geneva, Switzerland; [2] Service of Infectious Diseases, University Hospital and Medical School of Geneva, 4 rue Gabrielle-Perret Gentil CH-1205 Geneva, Switzerland; [3] School of Physics and Astronomy, The University of Manchester, M13 9PL Manchester, UK;

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ABSTRACT

Bacterial cells can resist an antibiotic treatment resulting in chronic bacterial infections. To survive a lethal dose of antibiotics, bacteria change their physiological state to a non-growing state, called dormancy. The mechanisms of entry into bacterial dormancy are not understood. Toxin-antitoxin system (TAS) is a stress-inducible functional unit that comprises a toxin component and a corresponding antitoxin that blocks toxin activity. In *E. coli*, overexpression of the toxin component leads to cell growth defects, suggesting that bacterial TASs can be involved in development of bacteria dormancy to resist antibiotic treatment. We are working with pathogen bacteria Staphylococcus aureus and investigating the role of an endoribonuclease MazF toxin on bacterial dormancy. We identified genome-wide RNA targets cleaved by MazF endoribonuclease and demonstrated that MazF overexpression causes S. aureus dormancy. To further understand MazF effect on *S. aureus* cell metabolism, we applied RNA-seq, RIBO-seq (ribosome profiling) and quantitative mass spectrometry. Our results show that activation of MazF toxin reduces ribosome biogenesis, translation, and induces formation of ribosome dimers, that decreases cell metabolism and provokes bacterial dormancy. Thus, our data suggests that MazF toxin may be a key regulator of dormancy and antibiotic resistance in S. aureus. To understand how MazF toxin can be activated under natural conditions, we investigated an upstream pathway regulating MazE antitoxin degradation. Our results suggest that environmental stress, such as heat shock, oxidative stress or high concentration of antibiotics induce aggregation of adaptor protein YjbH, that may result in activation of MazF toxin. We proposed the model (Figure 1) where S. aureus MazEF Toxin-Antitoxin System is modulated by adaptor proteins in response to environmental stress and regulates bacteria dormant state. Our model suggests a potential pathway to regulate antibiotic resistance in pathogens

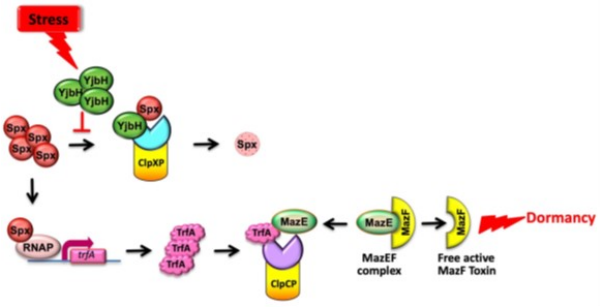

Figure 1 - In bacteria *S.aureus* MazEF Toxin-Antitoxin System regulates cell dormancy in response to environmental stress, such as heat shock, oxidative stress, and presence of antibiotics.

Correspondence: Email - olesya.panasenko@unige.ch

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Comprehensive metabolomics studies to identify biomarkers of type 1 diabetes onset and pancreatic islet stress

Peter Buchwald

Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL, USA

Available Online: 15 November 2019

ABSTRACT

Technological advances now allow quantitative assessment of biochemicals in small enough samples to make possible longitudinal studies involving multiple sample collections in rodents or small human tissue cultures. To investigate biochemical changes caused by onset of type 1 (juvenile-onset) diabetes and identify biomarkers of islet stress, we carried out a longitudinal metabolomics study in NOD mice, the most commonly used animal model of this diseases. Using complex LC-MS analyses for metabolomics profiling, we were able to quantify the concentration time-profile of more than 650 biochemicals in blood and feces [1]. In animals that became diabetic, several of them showed considerable (>4x) change, and some, such as 3-hydroxybutyrate (BHBA), maltose, and 1,5-anhydroglucitol, changed by more than 10-fold (Figure 1). While there were no strong differences between the metabolic signatures of progressors and non-progressors before the onset of diabetes, we found compounds that had significantly different levels and show promise as possible early biomarkers, maybe as part of a combined metabolic signature. In a separate study, we investigated the effects of stress factors such as inflammation and hypoxia on the biochemical profile of isolated human islets under both basal and hyperglycemic conditions [2]. Using metabolomics profiling of media and cell samples via the same method, we were able to identify and quantify a total of 241 and 361 biochemicals, respectively. One of the most notable observations was the consistently large changes induced by an inflammatory milieu in kynurenine and kynurenate, suggesting that they might serve as biomarkers of islet inflammation and that indoleamine-2,3-dioxygenase (IDO) on the corresponding metabolic pathway could be a therapeutic target to modulate the effects of inflammation in islets.

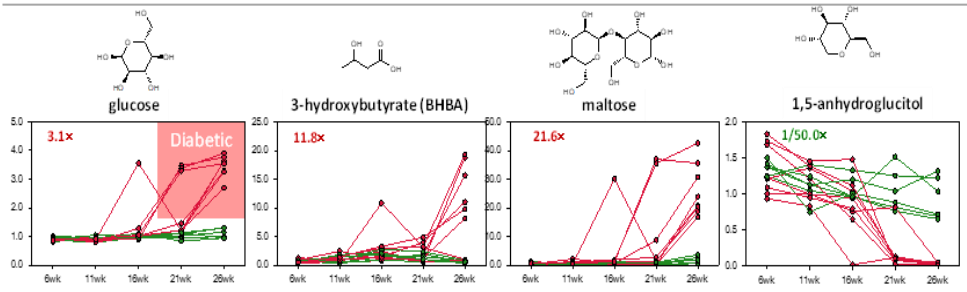

Figure 1 - Spaghetti plots illustrating the longitudinal time-profile of some of the small-molecule biochemicals showing the largest fold-change in diabetic progressor NOD mice (red) versus non-progressor controls (green) in blood samples. Individual data (scaled intensity) are shown with numbers indicating the average fold-change value at week 26, when all progressors were already diabetic.

Acknowledgments:

Financial support by the National Institutes of Health (NIH/NIDDK, 1UC4DK104208) and the Diabetes Research Institute Foundation is gratefully acknowledged.

References:

[1] Buchwald P, Tamayo-Garcia A, Ramamoorthy S, Garcia-Contreras M, Mendez AJ, Ricordi C. J Proteome Res 16 (2017) 3873-90.

[2] Garcia-Contreras M, Tamayo-Garcia A, Pappan KL, Michelotti GA, Stabler CL, Ricordi C, Buchwald P. J Proteome Res 16 (2017) 2294–306.

Correspondence: Email - pbuchwald@med.miami.edu

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Engineering bacterial monooxygenases to produce human drug metabolites

Sheila J. Sadeghi*, Gianfranco Gilardi

Department of Life Sciences & Systems Biology, University of Torino

Available Online: 15 November 2019

ABSTRACT

Flavin monooxygenases are a large family of enzymes carrying out oxidative reactions. One of these group of enzymes, Baeyer-Villger monooxygenases (BVMO), are wide spread in nature especially in bacteria and fungi but absent in humans. These enzymes are of great biotechnological interest due to their stereo- and regio-specific conversion of ketones into the corresponding esters and lactones, for green chemistry applications. Previously we have identified and cloned a BVMO (Ar-BVMO) from the genome of Acinetobacter radioresistens and found that it is closely related not only to a medically relevant monooxygenase (ethionamide-prodrug activator) [1] but also by itself capable of inactivating the imipenem antibiotic [2]. By multi-alignment of several known primary sequences of BVMOs, a conserved arginine thought to be located in the active site of these enzymes was also found to be present in Ar-BVMO. Since this conserved amino acid is crucial for the enzymatic activity of other BVMOs, we proceeded by replacing the latter arginine, R292 in Ar-BVMO (Fig. 1), with glycine or alanine. After expression and purification of the mutant proteins, their activities were compared to that of the wild type Ar-BVMO. As expected, the results obtained demonstrated the drastically reduced Baeyer-Villiger activities of both mutants. But somewhat unexpectedly and to our surprise, one of the two mutants, R292A, was still capable of heteroatom oxidation, reactions similar to those carried out by human hepatic drug metabolizing enzymes. For example, it could convert the anti-tubercular drug ethionamide to its corresponding S-oxide form (Fig. 1). In this way, the advantages related to stability and solubility of a bacterial enzyme are combined with the catalytic specificity of a human membrane-bound enzyme

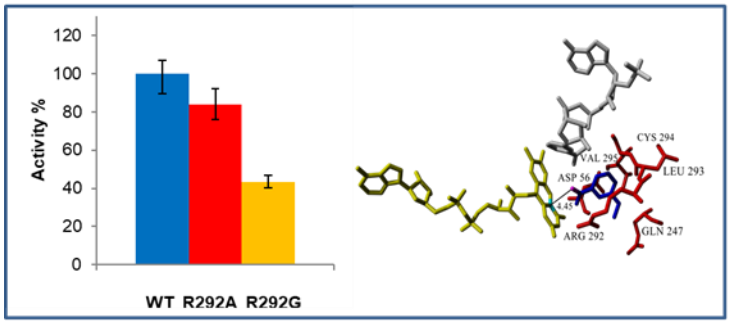

Figure 1 - Left: Graph showing the difference activities of the wild type and the two engineered mutants towards ethionamide (anti-tubercular drug); Right: 3D model of Ar-BVMO active site with flavin cofactor in yellow and ethionamide in dark blue.

References:

[1] D. Minerdi, I. Zgrablic, S.J. Sadeghi, G. Gilardi, Microbial Biotechnology 5 (2012) 700-716.

[2] G. Catucci, I. Zgrablic, F. Lanciani, F. Valetti, D. Minerdi, D.P. Ballou, G. Gilardi, S.J. Sadeghi, Biochim Biophys Acta. - Proteins and Proteomics 1864 (2016)1177-1187.

Correspondence: Email - sheila.sadeghi@unito.it

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Proteogenomic analysis of Mycobacterium tuberculosis Beijing B0/W148 cluster

J. A. Bespyatykh*, A. V. Smolyakov, G. P. Arapidi, E. A. Shitikov

Federal Research and Clinical Centre of Physical-Chemical Medicine, Moscow, Russia

Available Online: 15 November 2019

ABSTRACT

Today, the whole genome structure and it's most complete description, annotation, are the start points in the study of life organisms. At the same time experimental datasets obtained on proteomic and transcriptomic level can improve the annotation, providing evidence of new genes and correcting known ones. The aim of this study was to improve the annotation of RUS_B0 strain of M. tuberculosis Beijing B0/W148 cluster using the proteogenomic analysis. The resulting (circular) genome of RUS_B0 strain was obtained using Illumina HiSeq2500 platform and Sanger sequencing (GenBank accession number is CP030093.1). LC -MS/MS proteome analysis of 58 Beijing B0/W148 cluster strains was performed on a Q Exacitve HF mass spectrometer (Thermo Fisher Scientific, USA). According to proteomic data we identified 31,527 peptides corresponding to 2,782 proteins. For Genome Search Specific Peptides (GSSPs) identification six-frame translation of RUS_B0 genome carried out. According to proteogenomic analysis 67 GSSPs were identified. Among them 31 GSSPs were used for translation star site (TSS) correction of 32 annotated genes; 32 GSSPs - crossed along the coordinates with annotated pseudogenes and 4 GSSPs - corresponded to new, not annotated genes. Based on obtained data we confirmed the presence of peptides (n=32) for 8 RUS_B0 pseudogenes. Additionally cluster-specific singe amino acid polymorphism (Ala253Ser) in the oxalyl-CoA decarboxylase protein (TBPG_RS00635) was shown (Fig. 1). For GSSPs verification independent proteome analysis of two strains in two biological replicate was performed. According to identification of spectra obtained in targeted HR-MRM analysis performed on TripleTOF 5600+ mass-spectrometer 23 GSSPs were verified. The results allowed us to get the most complete annotation of RUS_B0 strain of Beijing B0/W148 cluster which is widespread in the Russia.

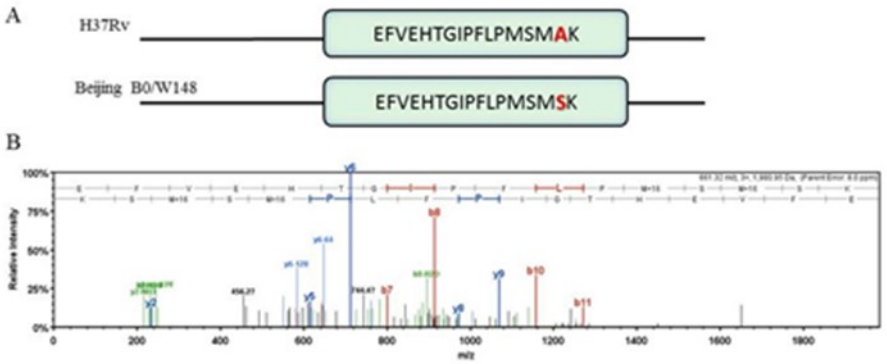

Figure 1 - Identification of Beijing B0/W148 cluster-specific SAP with representative MS/MS spectrum. A. The sequence of peptide which contains Beijing B0/W148 cluster-specific SAP and sequence of such peptide annotate in H37Rv. B. A representative MS/MS spectrum of peptide EFVEHTGIPFLPMSMSK.

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Correspondence: Email - sheila.sadeghi@unito.it

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Grapevine leaf proteome reveals specific adjustments leading Plasmopara viticola resistance

Andreia Figueiredo[1*], Joana Figueiredo[1,2], Rita B. Santos[1], Ana Rita Cavaco[1], Mónica Sebastiana[1], Ana Rita Matos[1], Anabela Silva[1], Marta Sousa Silva[2], Leonor Guerra-Guimarães[3], Jenny Renaut[4], Peter Roepstorff[5], Ana Varela Coelho[6]

[1] University of Lisboa, Faculty of Sciences, BioISI - Biosystems & Integrative Sciences Institute, Lisboa, Portugal; [2] Laboratório de FTICR e Espectrometria de Massa Estrutural & Centro de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa, Portugal; [3] Centro de Investigação das Ferrugens do Cafeeiro (CIFC) & Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia, Universidade de Lisboa, Oeiras, Portugal; [4] Luxembourg Institute of Science and Technology, Belvaux, Luxembourg; [5] Department of Biochemistry and Molecular Biology,University of Southern Denmark,Denmark. 6Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da Republica, Oeiras 2780-157, Portugal

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ABSTRACT

Grapevine downy mildew is an important disease affecting crop production leading to severe yield losses. We have conducted a leaf proteome study to identify specific adjustments on tolerant and susceptible grapevine genotypes to P. viticola (0h, 6, 12 and 24 hpi). Leaf proteome analysis was performed using 2D difference gel electrophoresis followed by protein identification via mass spectrometry. We have also accessed reactive oxygen species, antioxidant capacity, lipid peroxidation and gene expression. By analyzing the constitutive differences, proteins related to photosynthesis and metabolism allowed the discrimination of resistant and susceptible grapevine cultivars [1]. Following inoculation, increase of hydrogen peroxide levels, cellular redox regulation, establishment of ROS signalling and plant cell death seem to be key points differentiating the resistant genotype [1]. Lipid associated signalling events, particularly related to jasmonates appear also to play a major role in the establishment of resistance [1]. Aiming at a better understanding of the genotype-specific differences that account for a successful establishment of a defense response to the downy mildew pathogen we have developed an apoplastic fluid extraction method through vacuum infiltration compatible with both proteome and metabolome analysis. To perform this analysis, APF was extracted from susceptible and tolerant cultivars/genotypes, Trincadeira and Regent, respectively. Our preliminary results show that both genotypes are inherently different at the intracellular space composition. Label-free proteome sequencing by LC-MS approach will be conducted at several inoculation time-points in order to define the key events associated to pathogen recognition and signalling activation that lead to the establishment of the incompatible interaction.

Acknowledgments:

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References:

[1] A. Figueiredo, J. Martins, M. Sebastiana, A. Guerreiro, A. Silva, A. R. Matos, F. Monteiro, M. S. Pais, P. Roepstorff, A. V. Coelho. Journal of Proteomics (2017) 152 48–57.

Correspondence: Email - sheila.sadeghi@unito.it

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Exploration of the platelet proteomic signature associated with adverse transfusion reactions

Sandrine Laradi[1,2*], Danielle Awounou[1#], Chaker Aloui[1,2#], Celine Barlier[1#], Stephane Claverol[3], Jocelyne Fagan[1,2], Emmanuelle Tavernier[4], Christiane Mounier[4] Hind Hamzeh-Cognasse[2], Fabrice Cognasse[1,2], Olivier Garraud[1,5]

# These auteurs have contributed equally to this work

[1] French Blood Bank (EFS) Auvergne-Rhône-Alpes, Saint-Etienne, France; [2] GIMAP-EA3064, University of Lyon, Saint-Etienne, France ; [3] Proteome Platform, CGFB, University of Bordeaux Segalen, Bordeaux, France ; [4] Cancerology Institute Lucien Neuwirth, Saint-Priest en Jarez, France ; [5] National Institute of Blood Transfusion (INTS), Paris, France

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ABSTRACT

Grapevine downy mildew is an important disease affecting crop production leading to severe yield losses. We have conducted a leaf proteome study to identify specific adjustments on tolerant and susceptible grapevine genotypes to P. viticola (0h, 6, 12 and 24 hpi). Leaf proteome analysis was performed using 2D difference gel electrophoresis followed by protein identification via mass spectrometry. We have also accessed reactive oxygen species, antioxidant capacity, lipid peroxidation and gene expression. By analyzing the constitutive differences, proteins related to photosynthesis and metabolism allowed the discrimination of resistant and susceptible grapevine cultivars [1]. Following inoculation, increase of hydrogen peroxide levels, cellular redox regulation, establishment of ROS signalling and plant cell death seem to be key points differentiating the resistant genotype [1]. Lipid associated signalling events, particularly related to jasmonates appear also to play a major role in the establishment of resistance [1]. Aiming at a better understanding of the genotype-specific differences that account for a successful establishment of a defense response to the downy mildew pathogen we have developed an apoplastic fluid extraction method through vacuum infiltration compatible with both proteome and metabolome analysis. To perform this analysis, APF was extracted from susceptible and tolerant cultivars/genotypes, Trincadeira and Regent, respectively. Our preliminary results show that both genotypes are inherently different at the intracellular space composition. Label-free proteome sequencing by LC-MS approach will be conducted at several inoculation time-points in order to define the key events associated to pathogen recognition and signalling activation that lead to the establishment of the incompatible interaction.

Acknowledgments:

This work was supported by grants from the French National Blood Service–EFS (Grant APR 14 2016-32), France; the Association Recherche Transfusion (ART) (2018-19) and the Association "Les Amis de Remi", Savigneux, France. We thank the EFS delivery department of Saint-Priest-en-Jarez for their precious collaboration in the collection of PC bag sampling. We also thank the blood donors for agreeing to take part in this study.

References:

[1] ANSM, Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM), Hemovigilance activity report. (2018).

[2] C. Aloui, C. Barlier, S. Claverol, J. Fagan, D. Awounou, E. Tavernier, D. Guyotat, H. Hamzeh-Cognasse, F. Cognasse, O. Garraud, S. Laradi, J Proteomics (2019) 194 25-36.

Correspondence: Email - sandrine.laradi@efs.sante.fr

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Chromosome-centric proteomic QR-code: promising tool for personal health molecular monitoring and health analytics.

Elena A. Ponomarenko[1]*, Ekaterina V. Ilgisonis[1], Mikhail A. Pyatnitskiy[1], Andrey V. Lisitsa[1], Ekaterina V. Poverennaya[1], Arthur T. Kopylov[1], Victor G. Zgoda[1] and Alexander I. Archakov[1]

[1] Institute of Biomedical Chemistry (IBMC). 10 building 8, Pogodinskaya street, 119121, Moscow, Russia.

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ABSTRACT

Purpose: for the practical use of the International Chromosome-Centric Human Proteome Project (C-HPP) results in medicine, it is necessary to uncover which part of the human plasma proteome could be identified and measured.

Experimental description: within the framework of the C-HPP, the Russian Consortium is developing plasma analysis technology, which combines the chromosome-centric approach with bottom-up Selected Reaction Monitoring with Stable Isotope-labeled peptide Standards (SRM SIS). This study was aimed to quantitatively analyze the proteins encoded by 643 genes of the four selected chromosomes in the blood plasma of healthy, clinically well-examined people using SRM SIS technologies. Fifty-four male subjects (age 20-47) were examined at the Institute of Medico-Biological Problems (Moscow, Russia) and approved for space-related simulations and experiments.

Results: the concentration of 205 proteins was accurately measured with SRM SIS assay (1), while quantitative proteomic profile of each sample was presented as personal QR-code. The concentration range covered by the SRM SIS technology was six orders of magnitude (from 10^{-6} to 10^{-11} M) in case of the analysis of whole plasma, and five orders of magnitude (from 10^{-7} to 10^{-11} M) in case of the analysis of depleted samples. Unexpectedly, from the obtained data we derived no correlation between protein abundances and corresponding number of samples in which this protein was detected.

Conclusions: there are only about 30 proteins encoded by examined 643 protein coding genes, which abundance levels are more or less stable (inter-individual CV ≤20%). It seems that those proteins could be used as a pillar for creation SRM-assays for personal health analytics.

Acknowledgments:

This study was supported by the Program of the Presidium of the Russian Academy of Sciences ("Proteomic and Metabolomic Profile of Healthy Human"). Data storage and analysis was performed using equipment of "Human Proteome" Core Facility (Institute of Biomedical Chemistry). Ek.I. and Ek.P. acknowledge the Leading Scientific School of Prof. Andrey Lisitsa (grant the Russian Federation of President NSh -6313.2018.4)

References:

Kopylov AT, Ponomarenko EA, Ilgisonis EV, Pyatnitskiy MA, Lisitsa AV. et al., 200+ Protein Concentrations in Healthy Human Blood Plasma: Targeted Quantitative SRM SIS Screening of Chromosomes 18, 13, Y and the Mitochondrial Chromosome Encoded Proteome. J Proteome Res. 2018 Nov 27. doi: 10.1021/acs.jproteome.8b00391

Correspondence: Email - 2463731@gmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Computational approach to detect interference in SRM data

E.V. Ilgisonis[1]*, A.T. Kopylov[1], A.V. Lisitsa[1]

[1] Institute of Biomedical Chemistry (IBMC). 10 building 8, Pogodinskaya street, 119121, Moscow, Russia.

Available Online: 15 November 2019

ABSTRACT

Purpose: Proteomic technologies, particularly SRM target mass-spectrometry, enable to detect thousands of proteins in biological samples. These data require appropriate means for storage, analytical processing and interpretation to decipher the new knowledge. This work reviews modern state of target mass-spectrometry in proteomics. However, the most of SRM data is distorted by interference. The main reason of the interference is that some components in a complex biological sample may have the same precursor and fragment masses as the target peptide 1. Currently, manual inspection is used to find the interference. To formalize expert's inspection results we have developed an algorithm to detect the interference and to estimate its influence on the quantitative protein analysis.

Experimental description: In the present work we collected and normalized chromosome 18 protein detection SRM results. 2247 peptides of 275 chromosome 18 proteins were detected in human plasma and HepG2 cell line. Three SRM transitions for each peptide were monitored. Each experiment was repeated in 3 technical runs. For the selected peptides stable isotope labeled internal standard peptides was used. Data processing and interference detection were programmed in Perl. The measurements were segmented according to the within-run CV of transitions' intensity and peptides; measured concentrations. The most confident data were revealed by removing the transitions with CV>30%.

Results: We performed analysis of the huge SRM data set, including 275 proteins of chromosome 18. Data array was processed using cascade filters based on the analysis of mass-spectrometric parameters reproducibility and consistency. In total, 23 from 275 (chr.18) proteins were detected with high confidence. For the most confident and most variable changeable proteins we performed meta-analysis to find properties, that unite them.

Conclusions: In conclusion, we presented a method for detection of interference based on reproducibility in technical runs and technical replicates. SRM detection results are discrepant because of technical and biological variability. In addition, we showed, that the difference between measured concentrations of two peptides of one protein may be explained by the fact, that at least one of them conforms not only to the canonical isoform of the target protein, but to splice isoforms of other proteins too.

Key Words: proteomics, SRM, mass-spectrometry, bioinformatics, database

Acknowledgments:

This study was funded by the Russian Academy of Sciences (Fundamental Research Program for 2013–2020). Data storage and analysis was performed using equipment of "Human Proteome" Core Facility (Institute of Biomedical Chemistry). Ek.I. acknowledge the Leading Scientific School of Prof. Andrey Lisitsa (grant the Russian Federation of President NSh-6313.2018.4)

References:

[1] Sherman J, Molloy MP, Burlingame AL. Why complexity and entropy matter: information, posttranslational modifications, and assay fidelity. Proteomics. 2012;12(8):1147–50. doi:10.1002/pmic.201100459.

Correspondence: Email - ilgisonis.ev@gmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

HAX1 interactome mapping based on two complementary approaches reveals new functions in oxidative phosphorylation and protein aggregation

Mateusz Chmielarczyk[1]*, Maciej Wakula[1], Anna Balcerak[1] C, Ryszard Konopiński[1], Tymon Rubel[2], Ewa A. Grzybowska1

[1] Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland; [2] Warsaw University of Technology, Warsaw, Poland

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ABSTRACT

HAX1 protein is involved in the regulation of apoptosis, cell migration and calcium homeostasis, but the exact mechanisms of its action remain elusive. HAX1 expression level has medical implications; its deficiency causes severe neutropenia and neurological symptoms, while its overexpression is observed in several types of cancer [1]. In this study we aimed to elucidate HAX1 role in the cell by characterizing its protein interactome using two different approaches: yeast two-hybrid system (Y2H) and affinity purification coupled with mass spectrometry (AP-MS). The results revealed a substantial variability of the protein subsets, depending on the method and the specific cancer cell line used. Detailed analysis of the results using STRING protein association networks revealed a large subset of mitochondrial proteins involved in metabolic regulation, namely proteins of malate-aspartate shuttling system and respiratory chain proteins, possibly functioning in metabolic shift, observed in cancer cells. However, the strongest and the most reliable interaction partner, confirmed in all approaches and by additional co-immunoprecipitation and microscopic studies was the mitochondrial chaperone CLPB (caseinolytic peptidase B). CLPB belongs to the AAA superfamily of ATP-ases and is involved in the disaggregation of protein aggregates. Its deficiency is lethal, due to significant neonatal neurologic disorders and severe neutropenia, which partially conforms to the observed HAX1 deficiency symptoms. Thus, further analysis of these results should contribute to a better understanding of HAX1 functions and their medical implications.

Acknowledgments:

This work was supported by the Polish National Science Center grants no. 2011/01/B/NZ1/03674 and 2014/14/M/NZ1/00437

References:

[1] Fadeel, B. Grzybowska, E.A. Biochim Biophys Acta. 2009 Oct;1790(10):1139-48

Correspondence: Email - chmielarczykmateuszbiotech@gmail.com

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

A new insight into cryptobiosis: Cell repair in ametabolism

Yoichiro Sogame[1]*, Ryota Saito[1], Ryota Koizumi[1]

[1] Laboratory of animal physiology, National Institute of Technology Fukushima College, Japan

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ABSTRACT

Cryptobiosis is a physiological state of reversible ametabolism. During cryptobiosis, organisms show no visible signs of life; their metabolic activity becomes almost impossible to measure. Many organisms, for example African chironomids, tardigrades, brine shrimp eggs, and single-celled eukaryote protozoa such as Colpoda, undergo cryptobiosis. During cryptobiosis, they acquire extreme tolerance to stresses by halting their metabolism. Colpoda is a single-celled eukaryote protozoa adapted to terrestrial environments. Its strategy involves formation of a resting cyst (encystment) in response to desiccation stress. This encystment is a form of cryptobiosis. Therefore, they also possess the ability to revert to a vegetative state when the stressor has passed. In addition, they exhibit extreme tolerance to many environmental stresses, for example high and low temperatures, acids, organic solvents, and so on. In the processes of encystment and excystment, the expression of some proteins has been reported to be altered [1]. In general, cell injuries caused by gamma rays are either direct or indirect injuries; the latter are caused by ROS stress. In our study, we demonstrated that cell injuries caused by gamma ray irradiation were repaired during cystic state as cryptobiosis in C. cucullus R2TTYS strain [2]. In this presentation we will discuss cell repair during cryptobiosis.

Keywords: Cryptobiosis, Cyst, Colpoda

Acknowledgments:

This research was financially supported by JSPS KAKENHI (Grant Number: 16K18827, 19K16193), and by Sasakawa Scientific Research Grant (#24-407, #29-808).

References:

[1] Sogame Y., Kojima K., Takeshita T., Kinoshita E., Matsuoka T., 2014. Identification of differentially expressed water-insoluble proteins in the encystment process of Colpoda cucullus by two-dimensional electrophoresis and LC-MS/MS analysis. J. Eukaryot. Microbiol. 61: 51-60.

[2] Sogame Y., Saito R., Koizumi R., Shimizu T., Ono T. Evidence of stress recovery in free-living ciliate Colpoda cucullus: The repair capability of resting cysts to damage caused by gamma irradiation. Acta Protozool. (In press).

Correspondence: Email - sogame@fukushima-nct.ac.jp

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE VI INTERNATIONAL CAPARICA CONFERENCE ON ANALYTICAL PROTEOMICS 2019 (ICAP 2019)

Neutrophils response to surgery and ischemia from a proteomic standpoint

W. Fontes[1*], S. Arshid[1,2], M. Tahir[1,3], B. Fontes[2], E. F. S. Montero[2], M. S. Castro[1], S. Sidoli[3], V. Schwämmle[3], P. Roepstorff[3]

[1] Laboratory of Protein Chemistry and Biochemistry, Institute of Biology, University of Brasilia, Brazil; [2] Laboratory of Surgical Physiopathology (LIM-62), Faculty of Medicine, University of São Paulo, Brazil; [3] Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark

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ABSTRACT

Neutrophils play an important role in the mechanism of traumatic injuries, ranging from simple surgical traumas to polytrauma patients. The mesenteric ischemia/reperfusion (IR) model in laboratory animals is well suited to represent the systemic inflammatory events following severe trauma. The ischemic preconditioning (IPC), characterized by short IR events preceding a longer ischemia, shows a protective effect against the inflammatory damage. Although neutrophils are known to be key players in such systemic inflammatory response, the molecular mechanisms underlying their function is yet controversial and there are no viable treatments to modulate their response in patients. In the studies presented here we compared the proteomic profile of neutrophils from control rata and rats subjected to abdominal surgery (laparotomy), IR alone and IR preceded by IPC. After database searches, normalization and statistical analysis our proteomic analysis resulted in the identification of 2437 protein groups that were assigned to five different clusters based on the relative abundance profiles among the experimental groups. Cluster based KEGG pathway analysis revealed significant regulation of directional migration and chemotaxis after mild surgical trauma, added to phagocytosis and ROS production when the animals were exposed to IR. The inflammatory damage prevention by IPC was revealed at the molecular level to be related to related to chemokine signaling, transendothelial migration and, especially in the oxidative stress pathways (Figure 1). We identified interesting antioxidants including peroxiredoxin-6, glutathione peroxidase, and methionine sulfoxide reductase which were found downregulated after IR. Such a decrease in antioxidant activity in IR neutrophil leads to higher ROS production further contributing to tissue damage whereas IPC prevented such changes. Similarly another interesting enzyme, argininosuccinate synthase, involved in NO generation (nitrosative stress) was found significantly increased in IR and decreased in IPC.

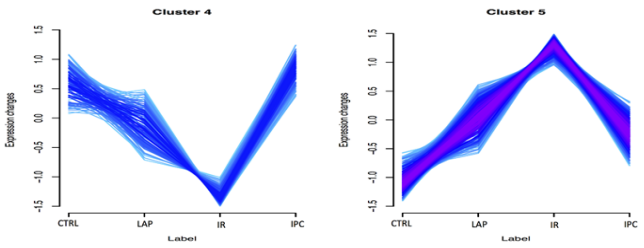

Figure 1 - Two clusters of regulated proteins showing the effect of surgery and IR, as well as the protective effect of IPC

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References:

[1] Tahir M et al. Front Mol Biosci. 2018;5:89.

[2] Arshid S. et al. Journal of Proteomics. 2017; 151:162-173.

[3] Arshid S. et al. Proteomics - Clinical Applications. 2017; 11(1-2):1600001-.

Correspondence: Email - wagnerf@unb.br

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One Enterocin AP-7121: combination with colistin against human multi-drug resistant Gram-negative pathogens

G. Delpech[1], S. Lissarrague[1], M. Ceci[2], L. García Allende[2], A. Lallee[1], B. Baldaccini[1], M. Sparo[1*]

[1] Microbiología Clínica, Medicina, Universidad Nacional del Centro de la Provincia de Buenos Aires, Olavarría (7400), Argentina;[2] Centro de Estudios Bioquímicos, Tandil (7000), Argentina.

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PRELIMINARY COMMUNICATION

The significant prevalence of Gram-negative bacteria as health-care associated pathogens and their increased antimicrobial multi-drug resistance highlight the need for new therapeutic options. Colistin is a conventional antimicrobial currently employed for the treatment of nosocomial infections caused by multi-drug resistant Gram negative bacteria such as Pseudomonas aeruginosa and Acinetobacter baumannii complex with a main drawback, its toxicity. Doses of this drug, and its toxic effects, can be potentially reduced by using it combined with bacteriocins. AP-7121 is an enterocin produced by the probiotic strain Enterococcus faecalis CECT7121. The aim of this study was to investigate the synergistic activity of AP-7121 combined with colistin against multi-drug resistant Gram-negative pathogens. P. aeruginosa (n: 3) only susceptible to colistin and A. baumannii complex (n: 3) only susceptible to colistin and tigecycline were included. These human isolates were recovered from blood cultures (hemoculture) of patients with catheter-related bloodstream infections at the Intensive Care Unit (Hospital Ramon Santamarina de Tandil Argentina). Minimum Inhibitory Concentration (MIC) for AP, colistin, and colistin/AP-7121 combination against Gram-negative bacteria was assayed (micro-dilution method, CLSI 2018). In vitro bactericidal activity of AP alone or combined with colistin (MIC/4), for assessing a synergistic effect, was studied carrying out time-kill curves. Samples were obtained for viable cell counts (0, 4, 8 and 24 h). MIC and time-kill curves were carried out three times, in duplicate. Results were expressed as their average values. All isolates were resistant to AP (MICAP-7121 > 128 mg/L). Colistin showed anti-P. aeruginosa (MICcolistin 0.5 mg/L) and anti-A. baumannii complex (MICcolistin 0.5-1.0 mg/L) activity in each isolate. Colistin/AP-7121 Combination showed bactericidal activity against P. aeruginosa (MICcolistin/AP-7121 ≤ 0.06/11-0.12/16 mg/L) and A. baumannii (MICcolistin/AP-7121 ≤ 0.12-0.20/16 mg/L). A synergistic effect (colistin/AP-7121) was observed at 4-8 and 24 h for P. aeruginosa (-1.8 to -3.8 Δlog10 CFU/mL) and for A. baumannii complex isolates (-2.0 to -3.8 Δlog10 CFU/mL). AP-7121 is a candidate as an alternative option for the combination with colistin, against human P. aeruginosa and A. baumannii complex isolates producers of bloodstream infections. Their synergistic activity against these bacteria, leads to a bactericidal activity of AP, with lower MIC values and a potential reduction of colistin toxicity, to be thoroughly investigated.

Keywords: enterocin, AP-7121, colistin, Gram positive, human, multi-drug resistant, pathogens

1. Introduction

The significant prevalence of Gram-negative bacteria as health-care associated pathogens and their increased antimicrobial multi-drug resistance highlight the need for new therapeutic options [1-3].

Among health-care associated infectious diseases, a significant problem for Public health are catheter-associated bloodstream infections, where Pseudomonas aeruginosa and Acinetobacter baumannii complex are reported as part of the commonest bacterial agents of these infections [4].

Previous studies showed that multi-drug resistant P. aeruginosa was considered as a risk factor for increased in-hospital mortality and 30-day mortality after infection, as well as the presence of catheters was considered a risk factor for colonization with this species. In the case of A. baumannii, the fatal outcome of infections has increased due to carbapenems resistance, with a mortality rate approaching to 60%, including bloodstream infections [2, 5].

In 2017, in Argentina, a nation-wide surveillance reported

Corresponding Author: M. Sparo, Microbiología Clínica, Medicina, UNCPBA. Av. Pringles 4375, CP 7400, Olavarría, Argentina. +54 249 4428797, E-mail: monicasparo@gmail.com

resistance to fluorquinolones (25%) and carbapenems (29%) in P. aeruginosa, while 76% of Acinetobacter spp. isolates showed carbapenems resistance [6].

Colistin is a conventional antimicrobial currently employed for the treatment of nosocomial infections caused by multi-drug resistant Gram negative bacteria such as P. aeruginosa and A. baumannii complex with a main drawback, its toxicity [7].

A strategy for the treatment and control of Gram-negative pathogens has been testing the synergistic effect between a conventional antimicrobial together with natural compounds as bacterial antimicrobial peptides [8]. Therefore, doses of colistin and its toxic effects could be potentially reduced by using this drug combined with a bacteriocin such as AP-7121.

AP-7121 (formerly MR99) is an enterocin produced by the probiotic strain Enterococcus faecalis CECT7121, a non-hemolytic, gelatinase negative strain recovered from a natural corn silage in Tandil District, Argentina, which recently has been sequenced. Moreover, it does not show antimicrobial multi-resistance, with Minimum Inhibitory Concentration (MIC)vancomycin 0.12 μg/mL, MICampicillin 0.5 μg/mL, MICgentamicin < 500 μg/mL, and MICstreptomycin < 1000 μg/mL. Physicochemical studies showed that AP-7121 presents heat-stability (1 h, 75 °C). Also, it is sensitive to proteolytic enzymes, detergents, and chelants; in addition, it is stable against the activity of enzymes such as DNase, RNase, amilase, glucuronidase, and lipase. Also, presents inhibitory activity among a wide range of pH values (4.0–8.0). The mechanism of action is mediated by formation of pores in bacterial membranes, followed by osmotic shock and cell lysis [9-11].

The aim of this study was to investigate the synergistic activity of AP-7121 combined with colistin against multi-drug resistant Gram-negative pathogens.

2. Material and Methods

During the period July-December 2018, human isolates were recovered from blood cultures (hemoculture) of patients with catheter-related bloodstream infections at the Intensive Care Unit (Hospital Ramon Santamarina, Tandil Argentina). Phenotypic characterization was performed with conventional tests. For A. baumannii characterization was carried out to the complex level [12]. Disk diffusion susceptibility tests were done following Clinical and Laboratory Standards Institute`s guidelines [13].

P. aeruginosa (n: 3) only susceptible to colistin and A. baumannii complex (n: 3) only susceptible to colistin and tigecycline were included. Each bacterium was recovered from different patients, and one representative isolate was considered from each patient.

Phenotypic characterization and antimicrobial susceptibility to colistin (P. aeruginosa, A. baumannii complex) and tigecycline (A. baumannii complex) were validated with the Vitek 2 CompactTM automated system (bioMérieux, Buenos Aires, Argentina).

Isolation of AP-7121 was carried out according to a previously standardized protocol [14]. Probiotic strain E. faecalis CECT7121 (deposited at the Spanish Collection of Type Cultures, CECT, Burjasot, Valencia, Spain) was incubated in brain-heart infusión (BHI) broth (Laboratorio Britania, Buenos Aires, Argentina) at 35 ± 2 °C for 18 h. This culture was inoculated in 4 L of BHI broth and incubated at 35 ± 2 °C for 9 h.

Then, it was centrifuged at 15,000g, 4 °C, for 20 min. Supernatant was adjusted to pH: 7.0 and precipitated. After centrifugation at 20,000g, 4 °C, for 20 min, the pellet was re-suspended in 40 mL of phosphate buffer saline (PBS), pH: 7.0 (50 mM). AP-CECT7121 was isolated by physicochemical extraction employing Sep-Pak™ C18 cartridges (Waters, Milford, MS, USA). E. faecalis extract (5.0 mL) was loaded into a cartridge, previously washed with acetonitrile in trifluoroacetic acid (TFA, 0.1%), and it was eluted with acetonitrile (60%)-TFA (0.1%).

Eluate was concentrated to dryness using a vacuum centrifuge (Thermo Savant Instuments, Hollbrook, NY, USA). The obtained pellet was re-suspended in PBS (250 μL). Aliquotes (20 μL) of the suspensión were injected in a reverse-phase HPLC system (Shimadzu, Kyoto, Japan) and separated in a Nucleosil C18 (5 μm, Pharmacia, Uppsala, Sweden) column. Mobile phase: buffer A (TFA 0.1%) and buffer B (acetonitrile 95% in TFA 0.1%). AP-7121 was eluted using a linear gradient (95% A/5% B to 15% A/85% B), with a flow rate of 0.2 mL/min, controlling elution with a UV detector.

Fractions were collected at regular time period. Then, the active fraction was evaporated to dryness and re-suspended in phosphate buffer (50 mM, pH: 7.0). Biological activity of AP-7121 was detected in the eluate fractions after 30 min of the sample injection, when it was ca. 40% of buffer B.

Minimum Inhibitory Concentration (MIC) for AP-7121, colistin and the colistin/AP-7121 combination against P. aeruginosa and A. baumannii complex isolates was assayed with the broth micro-dilution method, according to the Clinical and Laboratory Standards Institute`s recommendations [13]. P. aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 were employed as quality control strains.

In vitro bactericidal activity of AP-7121 alone or combined with colistin (MIC/4), for assessing a synergistic effect, was studied carrying out time-kill curves. Fresh cultured bacterial cells were washed, suspended, and diluted in PBS, 50 mM, pH: 7.0, for reaching a 106 CFU inoculum. Samples (100 μL) of each bacterial suspension were obtained at 0, 4, 8, and 24 h of incubation (35 ± 2 °C). Viable colony counts were performed in BHI agar, after incubation at 35 ± 2 °C for 24 h. A viable cell count in the same experimental conditions, with PBS, was performed as quality control [14].

MIC and time-kill curves were carried out three times, in duplicate. Results were expressed as their average values, in mg/L for MIC and Δlog10 CFU/mL for viable counts.

3. Results

3.1. Bactericidal activity of colistin, AP-7121 and colistin/AP-7121 combination against human P. aeurginosa and A. baumannii complex isolates

All the studied P. aeruginosa and A. baumannii complex isolates were resistant to AP-7121, with MICAP-7121 > 128 mg/L (Table 1).

Anti-P. aeruginosa and anti-A. baumannii complex activity in each isolate, MIC: 0.5 mg/L and MIC: 0.5-1.0 mg/L, was detected respectively, for colistin (Table 1).

Combination of colistin and AP-7121 showed bactericidal activity against P. aeruginosa (MICcolistin/AP-7121 ≤ 0.06/11-0.12/16 mg/L) and A. baumannii complex (MICcolistin/AP-7121 ≤ 0.12-0.20/16 mg/L).

Table 1: Location Minimum Inhibitory Concentrations for AP-7121, colistin and colistin/AP-7121 against Pseudomonas aeruginosa and Acinetobacter baumannii complex from blood of patients with catheter-related infections.

Pseudomonas aeruginosa			
MIC	AP-7121	Colistin	Colistin/AP-7121
CEBUTI428	> 128	0.5	0.12/16
CEBUTI571	> 128	0.5	0.12/16
CEBUTI783	> 128	0.5	≤ 0.06/11
***Acinetobacter baumannii* complex**			
CEBUTI463	> 128	0.5	0.12/16
CEBUTI656	> 128	0.5	0.12/16
CEBUTI802	> 128	1.0	0.20/16

3.2 Assessment of early and late synerfystic effect of colistin/AP-7121 combination

A synergistic effect when colistin and AP-7121 (Table 2) were combined, it was observed at 4-8 h (early synergy) and 24 h for P. aeruginosa (-1.8 to -3.8 Δlog10 CFU/mL) and for A. baumannii complex (-2.0 to -3.8 Δlog10 CFU/mL).

4. Discussion

In this study, the synergistic activity of AP-7121 combined with colistin against human multi-drug resistant P. aeruginosa and A. baumannii complex isolates was investigated.

Worldwide, catheters-related infections are considered as one of the main causes of bloodstream infections associated with significant patient morbidity and mortality and increased health care costs [15]. Multi-drug resistance bacteria have become a critical risk factor for patients with bloodstream infections due to the limited therapeutic options. A French multi-center study showed that optimizing bloodstream infections management by increasing rapidity of appropriate treatment initiation may decrease short-term mortality, when patients received at least one active antibiotic within the first 48 hours [16].

In this sense, P. aeruginosa and A. baumannii complex constitute representative bacterial agents of catheter-related bloodstream infections due to their prevalence and their tendency to express multi-drug resistance and can be considered as bacterial models for the study of new alternatives for the treatment of these infections, namely bacteriocins such as enterocin AP-7121.

All the assayed isolates showed resistance to AP-7121 when it was not combined with colistin. Previously, enterocin AP-7121 presented homogeneous bactericidal activity against phylogenetically related bacterial species, i.e. Gram positive pathogens, from different origin, such as Enterococcus spp., Streptococcus spp., Staphylococcus aureus and Listeria monocytogenes but it was bacteriostatic against Gram negative bacteria, when it was assayed alone [9, 10].

Similar results were obtained by other authors when a bacteriocin was assayed against different bacteria. Garvicin KS, a broad-spectrum bacteriocin produced by Lactococcus garvieae, is effective against Gram positive and Gram negative bacteria. Nevertheless, this bacteriocin showed no bactericidal activity against P. aeruginosa isolates when it was tested alone. In addition, a lack of inhibition against A. baumannii isolates was observed for the lantibiotic nisin when it was not combined with other antimicrobial or bacteriocin [8].

When AP-7121 and colistin were assayed together against the bacteria, the determined MICs values were lower for the antimicrobial and significantly lower for the bacteriocin, compared with the obtained MICs for colistin and AP-7121

Table 2: Location Minimum Inhibitory Concentrations for AP-7121, colistin and colistin/AP-7121 against Pseudomonas aeruginosa and Acinetobacter baumannii complex from blood of patients with catheter-related infections.

Isolate	**Δlog_{10} CFU/mL**			
P. aeruginosa	0 h	4 h	8 h	24 h
Control	0	1.3	2.4	3.5
AP-7121	0	1.4	2.3	3.2
Colistin	0	-0.4	-1.2	-3.1
Colistin/AP-7121	0	**-1.8**	**-2.6**	**-3.8**
***A. baumannii* complex**				
Control	0	1.1	2.2	3.1
AP-7121	0	1.0	2.4	3.3
Colistin	0	-0.5	-1.4	-3.0
Colistin/AP-7121	0	**-2.0**	**-2.9**	**-3.8**

alone, showing a bactericidal effect achieved with the combination antimicrobial/bacteriocin for the studied isolates.

A traditional strategy for the treatment of infections with multi-resistant P. aeruginosa or A. baumannii is based on the combination of conventional antimicrobials in order to decrease the MIC values and be effective. A previous study assessed the efficacy colistin in combination with three different antimicrobials. Even though a drop of MICs was observed when compared to the ones for the individual antimicrobials, combinations were effective only in 13-20% of the resistant isolates while in most cases there was an additive/indifferent effect [17].

In addition, there was a lower fold decrease of MIC values for the combinations of colistin with other antimicrobials than the obtained with the combination colistin/AP-7121.

Combination of two or more conventional antimicrobials for the treatment of multi-drug resistant infections has another disadvantages compared to the use of a bacteriocin-antimicrobial combination. Resistance to new antimicrobials already emerged and together with an inadequate and non-controlled use of these drugs might contribute with a higher increase in resistance. The emergence and widespread of resistance of new antimicrobials is a significant drawback for therapy of these infections since might delay active treatment in patients with severe infections [7].

Decreased MIC values of the combination colistin-AP-7121 in the assayed human bloodstream P. aeruginosa and A. baumannii complex needs to be highlighted not only for contributing with less selective pressure for the emergence and spread of colistin resistance. Also, these results reinforce the possibility that a colistin-bacteriocin combination could potentially lead to a reduction of the toxic effects of colistin, as it was reported when this antimicrobial was assayed together with nisin [18].

The observed MICs reduction needs to be highlighted from the microbiological and therapeutical points of view, considering that nowadays colistin is still considered as one of the first-line treatment options for multi-drug resistant isolates of these bacterial species [7, 19].

Also, an early (4-8 h) and late (24 h) synergistic effect of the combination between colistin and AP-7121 was detected for P. aeruginosa and A. baumannii complex isolates in this study.

Recently, the synergistic effect of AP-7121 combined with conventional antimicrobials such as gentamicin and vancomycin, against Gram positive pathogenic bacteria was achieved [14]. Furthermore, when AP-7121 was assayed together with colistin for Gram negative bacteria of food origin (ground-beef Escherichia coli), bactericidal activity and a synergy were proven. These results suggested that the detergent effect of colistin against the outer membrane of Gram negative bacteria allowed AP-7121 to form pores that would lead to an osmotic shock followed by cell death, as it was previously observed for the enterocin against Gram positive bacteria [10, 20].

Other authors studied the possible combinations of an antimicrobial, polimixin B, with two bacteriocins, nisin and garvicin KS, with different results for each scheme. When there were assayed against A. baumannii, synergy was observed for garvicin KS-polimixin B but not for nisin-polimixin B mixtures, showed by the prevention or failing of bacteria regrowth. When the antimicrobial was tested together with both bacteriocins, they showed, as AP7121 did, an early synergistic effect after 4 h. However, there was no synergistic effect detected against P. aeruginosa when combinations of polimixin B with the two bacteriocins [8].

5. Concluding Remarks

According to the in-vitro obtained results, AP-7121 could be a candidate as an alternative option for the combination with colistin against human P. aeruginosa and A. baumannii complex isolates producers of bloodstream infections.

Their synergistic activity against these bacteria, leads to a bactericidal activity of AP7121, with lower MIC values and a potential reduction of colistin toxicity.

Further in vitro and in vivo studies need to be conducted in order to achieve a more comprehensive and thorough knowledge about the toxicity reduction effect over colistin, as well as the potential future availability and application of AP7121 in combination with this antimicrobial as a complementary tool for the treatment of these severe infectious diseases caused by multi-resistant Gram negative bacteria, and consider it as basic step for extending its usefulness for the prevention or treatment of other kinds of human infections.

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References

[1} B. Isler, Y. Doi, R.A. Bonomo, D.L. Paterson, Antimicrob Agents Chemother 63 (2018) e01110–e01118.
[2] L. Nguyen, J. Garcia, K. Gruenberg, C. MacDougall, Curr Infect Dis Rep 20 (2018) 23.
[3] R. Gahlot, C. Nigam, V. Kumar, G. Yadav, S. Anupurba, Int J Crit Illn Inj Sci 4 (2014) 162-167.
[4] Red WhoNet Argentina (2018). http://antimicrobianos.com.ar/2018/?cat=10.
[5] M. Bassetti, A. Vena, A. Croxatto, E. Righi, B. Guery, Drugs Context 7 (2018) 212527.
[6] M. Bassetti, P. Peghin, M. Vena, D.R. Giacobbe. Front Med (Lausanne) 6 (2019) 74.
[7] H. Chi, H. Holo, Curr Microbiol 75 (2018) 272-277.
[8] G. Delpech, N. García Allende, S. Lissarrague, M. Sparo, Open Infect Dis J 10 (2018a) 79-87.
[9] G. Delpech, M. Bistoletti, M. Ceci, S. Lissarrague, S.S. Bruni,

M. Sparo, Probiotics Antimicrob Proteins 9 (2017) 355-362.
[10] G. Delpech, E.M. Hebert, M. Sparo, L. Saavedra, Microbiol Resour Announc 8 (2019) e00245-19.
[11] M. Sparo, (2006a) Ph.D. thesis. University of Buenos Aires, Argentina.
[12] M.D. Sparo, M.S. Castro, P.J. Andino, M.V. Lavigne, C. Ceriani, G.L. Gutiérrez, M.M. Fernández, M.C. De Marzi, E.L. Malchiodi, M.A. Manghi, J Appl Microbiol 100 (2006b) 123-134.
[13] A.M. Leber, ed., Clinical Microbiology Procedures Handbook, fourth ed., ASM Press, Washington DC, 2016..
[14] Clinical and Laboratory Standards Institute. CLSI (2018) M100-S28.
[15] National Nosocomial Infections Surveillance System. National Nosocomial infections surveillance (NNIS) system report, data summary from January 1992 through June 2004. Am J Infect Control. 2004;32:470–485.
[16] O. Robineau, J. Robert, C. Rabaud, J.P. Bedos, E. Varon, Y. Péan, R. Gauzit, S. Alfandari, Infect Drug Resist 11 (2018) 1259-1368.
[17] H. Lee, K.H Roh, S.G. Hong, H.B. Shin, S.H. Jeong, W. Song, Y. Uh, D. Yong, K. Lee, Ann Lab Med 36 (2016) 138-144.
[18] G. Delpech, M, Ceci, L. García Allende, M. Sparo, J Vet Pharm Therap 41, Suppl 1 (2018b) 126.
[19] K. Naghmouchi, J. Baah, D. Hober, E. Jouy, C. Rubrecht, S. Famara, D. Drider, Antimicrob Agents Chemother 57 (2013) 2719-2725.
[20] N. Martis, S. Leroy, V. Blanc, J Infect 69 (2014) 1-12.

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One Health Approach for Identification of Sources/Reservoir of Multidrug Resistant Bacteria in Wild Animals and their Environment

Marilyn C. Roberts[1]*, Daira Melendez[1], Alexander L. Greninger[1], Samuel Wasser[1], Randall C.Kyes[1], Stefan Monecke[2], Ralf Ehricht[2], Prabhu Raj Joshi[3]

[1] University of Washington, Seattle WA USA; [2] Leibniz Institute for Photonic Technologies (Leibniz-IPHT), Jena Germany; [3] Nepalese Farming Institute, Maitidevi, Kathmandu Nepal.

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Bacteria such as extra-intestinal pathogenic E. coli (ExPEC) and methicillin-resistant S. aureus (MRSA) are important opportunistic pathogens. They might belong to pandemic, epidemic and/or sporadic clones. Some of the clones are associated with humans, others are associated with wild and/or domestic animals. Some clones are shared by both and may be found contaminating the environment. In these studies, we examined the spread of ExPEC from feces of Southern Resident Killer Whale (SRKW; Orcinus orca) that are associated with human diseases. We also examine MRSA isolates in wild rhesus macaques (Macaca mulatta), their environment and from humans. This One Health Approach aims to better understand the sources/reservoirs and possible transmissions of potential pathogens between animals, humans and their shared environment.

Keywords: Antibiotic resistance genes, extra-intestinal pathogenic E. coli, killer whales, MLST, MRSA, rhesus macaques

1. Introduction

Antibiotic resistant bacteria are identified in wild animals from birds to insects with increasing frequency [1]. The occurrence of these bacteria is often associated with human influence on the environment, spreading from people to animals and the environment or on occasion from animals or the environment back to people. The sharing of these antibiotic resistant bacteria has been documented primarily in land animals and birds, with limited work on marine mammals or primates [1]. In the current studies, we first examine the presence of E. coli from Southern Resident Killer Whales (SRKW), which are apex predators. This cetacean serves as a sentinel for its environment, providing valuable indices of the overall health of the Salish Sea (Puget Sound) boundary waters shared by Washington State, USA and British Columbia, Canada [4, 5]. The second study characterizes methicillin resistant Staphylococcus aureus (MRSA) isolated from wild rhesus macaques (Macaca mulatta) living in and around temple areas of the Kathmandu valley in Nepal, where human-macaque interaction is common, along with the shared environment in the valley and a few clinical strains from a local hospital.

2. Material and Methods

2.1. E. coli and DNA.

E. coli was isolated from fresh fecal samples collected from the endangered SRKW population (*O. orca*) in the Salish Sea [Puget Sound] in 2013. The red dots in Figure 1 indicate sampling sites off the San Juan Islands. Sample collection methods were approved by the University of Washington's Institutional Animal Care and Use Committee (IACC) under protocol 2850-08. Trained dogs were deployed on boats to detect specific SRKW fecal scent from distances farther than a nautical mile [4]. Samples were collected as part of a previous study and centrifuged into a small pellet on the boat [4]. Sterile Fisher Brand cotton swabs (Fisher Scientific Waltham, MA) were inserted into the homogenized faecal pellet and

Corresponding Author: 1959 NE Pacific St. Department of Environmental and Occupational Health 357234, School of Public Health, University of Washington, Seattle, WA USA Tel: 001-206-543-8001. Email: marilynr@uw.edu

Figure 1: Location of whale samples. Red dots show where fecal samples were collected.

~0.5 mL of each sample was removed and stored in 10 mL of sterile peptone water on ice. Samples were returned to the laboratory within 2-6 h of collection, vortexed for 10 seconds and 0.1 mL was spread on MacConkey agar plates (Difco Laboratories, Sparks, MD) supplemented with and without antibiotics including: 25 mg/L tetracycline, 25 mg/L chloramphenicol, or 25 mg/L ampicillin and incubated at 36.5 oC for 24-48 h. Nine of the eleven samples tested positive for E. coli, and 8 of the 9 grew on DifcoTM Luria-Bertani media (Difco) supplemented with 25 mg/L tetracycline [5].

E. coli isolates from different samples and plates were identified using standard biochemical tests. No E. coli were detected on either the ampicillin or chloramphenicol supplemented media. No E. coli could be isolated from 74 freeze-dried frozen faecal samples stored for >1 year. DNA extraction was done using MoBio Laboratories UltraClean® Microbial DNA Isolation Kit (Mo-Bio Laboratories, Carlsbad, CA.). The kit is designed to yield high-quality DNA from a variety of microbial isolates. Extracted DNA concentration was determined by using a Qubit (Thermofisher Technologies Inc., USA). Dual-indexed libraries were prepared using Nextera XT library prep kit (Illumina, San Diego, CA) with 1 ng of bacterial DNA and 14 amplification cycles. The kit used an engineered transposon with specific dual-indexed adapters that tagment the DNA during sequencing. The libraries were sequenced using an Illumina MiSeq. Raw-reads were trimmed using trimmomatic for quality, and de-novo assembled using SPAdes Genome Assembler v3.11 (Trimmomatic & SPAdes). Prokka v 1.13 was used to annotate genomes (prokka url). Assembled sequence data was deposited into NCBI GenBank under project PRNJNA338014 [5].

2.2. Isolation of MRSA and characterization.

A total of 227 saliva samples from wild rhesus macaques (Macaca mulatta) living in and around temple areas of the Kathmandu valley were collected during Feb 2018. The areas; Bajrayogini, Nilbarahi, Pashupatinath, Swyambhu and Thapathali were sampled with Bajrayogini being the most distant site from the city of Kathmandu. The collection technique involved an adaptation of the non-invasive oral sampling method in Evans [2] and Roberts [10] using SalivaBio Children's Swabs (Salimetrics LLC, State College PA, USA). Swabs were soaked in a sterile glucose solution (10% w/v) and thrown to the macaques, which they then chewed and discarded for immediate collection. Macaque saliva samples (n=13) and environmental samples (n=19) isolated near temple areas in Kathmandu were included in the study. Environmental surface samples (n=218) were collected in July 2018 from Bajrayogini, Nilbarahi, Pashupatinath, Swyambhu and Thapathali. High touch surfaces were selected at the temple sites. Solid surfaces were swabbed with sponges to collect the bacteria on the surfaces as previously described [6]. Five random isolates from wound infected patients were obtained by some of the Nepali authors for comparison with the primate and environmental MRSA. Beside their methicillin resistance, nothing was previously known about these isolates. Ethical approval was also obtained from the Kist Medical College and Teaching Hospital, Imadol, Lalitpur, Nepal, for the clinical MRSA isolates. All 37 isolates were characterized using The Alere StaphyType® DNA microarrays [7, 8]. The Abbott StaphyType® DNA microarray based assay was used for all isolates as previously described [7, 8]. The microarray typing includes 334 target sequences and ~170 separate genes and allelic variants including species markers, SCCmec, capsule, agr group typing markers, common antibiotic resistance genes, toxins and microbial surface components recognizing adhesive matrix molecules [MSCRAMM] genes [8].

3. Results

Whale E. coli. Nine distinct isolates were recovered and analyzed from seven SRKW individuals with whole genome sequencing, de novo assembly. Eight samples had multidrug resistant ExPEC ST73 clonotype C24:H10 isolates taken from 7 individuals from 3 pods (Table 1). The ninth isolate was not antibiotic resistant and was ExPEC ST127 clonotype C12:H2. All isolates carried a variety of virulence genes which differed between the ST73 isolates and between the ST73 and the ST127 isolates (Table 2). Previous studies showed that the Puget Sound (Salish Sea), home to the SRKW, is contaminated with multiple ARGs and antibiotic residues, especially near waste water treatment plant discharge sites [9]. The SRKW food source, Chinook salmon, also carry antibiotic residues in their tissue.

Nepalese MRSA. From the 227 primate saliva samples, 13 (5.7%) were MRSA positive. Multiple positive primate samples were identified in four of the five areas sampled: Bajrayogini [n=4], Pashupatinath [n=3], Swyambhu [n=2] and Thapathali [n=4]. In contrast, from the 218

Table 1: LST, Clonotype, Antibiotic Resistance of Whale E. coli. 4-UK* this may be a transient whale.

Isolate ID	ST	Clonotype	Resistance Genes	Predicted Phenotypes
1-J28	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
2-J28	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
3-J8	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
4-UK*	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
5-L79	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
6-J26	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
7-J27	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
9-J31	ST73	C24:H10	aadA1,sul1, tet(B)	Aminoglycoside, Sulfonamides, Tetracycline
8-J31	ST127	C12:H2	N/A	N/A

Table 2: Virulence factors Whale E. coli

Isolate ID	Adhesins	Toxins	Siderophores	Serum survival	Misc.
1-J28	iha, papC, sfaS	sat, ,hlyA	iutA, fyuA	iss	kpsM, malX
2-J28	iha, papC, sfaS	sat, vat, pic	iroN, ireA, iutA, fyuA	iss, ompT	kpsM, malX
3-J8	iha, papC, sfaS	sat, vat, pic	iroN, ireA, iutA, fyuA	iss	kpsM, malX
4-UK	iha, papC, sfaS	sat, vat, pic	iroN, ireA, iutA, fyuA	iss	kpsM, malX
5-L79	iha, papC, sfaS	sat, pic	iroN, ireA, iutA, fyuA		kpsM, malX
6-J26	iha, papC, sfaS	sat, pic	iroN, ireA, iutA, fyuA	iss	kpsM, malX
7-J27	iha, papC, sfaS	vat, pic, hlyA	iutA, ireA, fyuA		kpsM, malX
9-J31	iha	sat, vat, pic	iroN, ireA, iutA, fyuA	iss	kpsM, malX
8-J31	sfaS	cnf1, vat	iroN, ireA, fyuA	Iss, ompT	kpsM

environmental samples, 19 (8.7%) were MRSA positive cultured from all five areas sampled; Bajrayogini [n=3], Nilbarahi [n=2], Pashupati [n=6], Swyambhu [n=5] and Thapathali [n=3]. All 37 MRSA isolates were further characterized.

Twenty-three (62%) MRSA were CC22 SCCmec type IVa previously found in Nepalese macaque of human origin and isolated from monkey (n=4; 31%), environmental (n=14; 74%), and human (n=5; 100%) samples [10]. Eight monkey MRSA were CC361 SCCmec type IVa. One MRSA isolated from a monkey and environment were CC88 SCCmec type V, previously found in Nepalese swine samples [10]. The remaining environmental MRSA included one each, CC121 SCCmec type V, and CC772 SCCmec type V, all of human origin and two CC779 SCCmec type V, potentially a novel clone. All 37 MRSA carried the bla gene, 31 carried the aacA -aadD, 25 dfrA and 21 erm(C) genes. All CC22 isolates carried the aacA-aadD, dfrA and 17 carried the erm(C) genes, while 2 MRSA from macaque, 3 MRSA from environmental and 1 human MRSA lacked the erm(C) gene. The one macaque and environmental CC88 MRSA both carried the aacA-aphD gene but only the macaque MRSA carried the aphA3 and sat resistance genes, neither previously identified in primate MRSA [10] (Table 3). Among the 23 CC22 MRSA, 21 carried the PVL locus and tst virulence gene which is unusual and include all the monkey and human isolates and 12 of 14 environmental isolates [3]. This current study suggests that humans are the source of the MRSA identified in both the macaques and the environment and may be linked to humans feeding the primates and/or the primates living in close proximity to the humans.

4. Conclusions

As human populations continue to expand, so do opportunities for transmission of pathogens between humans and wildlife. We documented such transmission in a marine mammal and Old World terrestrial primate.

The study on antibiotic resistant E. coli isolated from SRKW helps to advance our understanding of the spread of AMR E. coli in the Salish Sea. It also demonstrates the need for increased microbial surveillance efforts of the declining SRKW population. Previous studies on the ST73 and ST127 have been associated with disease in humans and companion animals; however, without proper veterinary assessments, or urine samples it was not possible to determine if the whales were sick at the time of fecal collection. Therefore it is unknown if carriage of ExPEC isolates increases the risk of disease in the SRKW and/or if they contribute to the ongoing decline of this endangered species. This is the first time it was determined that Orca whales can carry antibiotic resistant potentially pathogenic strains of E. coli. The ExPEC isolates in the SRKW most

Table 1: MRSA strains, SCCmec types and resistance genes[a]. [a] The table shows only genes which were found at least once in this study. Genes which were not present in any of the study strains are: mecC, blaZ SCCmec XI, erm(A), erm(B), lnu(A), mef(A), vat(A), vat(B), vga (A), vga(A), vgb(A), far1, fusC, mupR, tet(K), tet(M), cat, cfr, fexA, qacA, qacC , vanA, vanB and vanZ.
[b] Subtypes are referred to by designations of reference strains that yield identical SCC patterns on the arrays used. GenBank references for these reference strains are as follows: MW2, BA000033.2; IS-105, AHLR; Bengal Bay (CMFT1723), HF569096.1; GR1, AJLX.

Strain	Host	N	mecA	SCCmec subtype[b] (number subtyped)	blaZ	erm(C)	msr(A)	mph(B)	aacA-aphD	aadD	aphA3	sat	dfrA
CC22-MRSA-IV (PVL+/ tst1+)	Rhesus	4	4	IVa as in MW2 (1)	4	2	-	-	4	-	-	-	4
	Environment	12	12	IVa as in MW2 (1)	12	9	-	-	12	-	-	-	12
	Human	5	5	IVa as in MW2 (2)	5	4	-	-	5	-	-	-	5
CC22-MRSA-IV (tst1+)	Environment	1	1	IVa as in MW2 (1)	1	-	-	-	1	-	-	-	1
CC22-MRSA-IV (PVL)	Environment	1	1	IVc as in IS-105 (1)	1	-	-	-	1	1	-	-	1
CC88-MRSA-V	Rhesus	1	1	V as in Bengal Bay (1)	1	1	1	1	1	-	1	1	-
CC88-MRSA-V (PVL+)	Environment	1	1	V as in Bengal Bay (1)	1	1	-	-	1	-	-	-	-
CC121-MRSA-VT	Environment	1	1	VT as in GR1 (1)	1	1	-	-	1	-	-	-	-
CC361-MRSA-IV	Rhesus	8	8	IVa as in MW2 (1)	8	-	-	-	-	-	-	-	-
CC772-MRSA-V (PVL+)	Environment	1	1	V as in Bengal Bay (1)	1	-	1	1	1	-	1	1	-
CC779-MRSA-VT	Environment	2	2	VT as in GR1 (1)	2	2	-	-	2	-	-	-	-

likely are either directly acquired from pollution in the Salish Sea, and/or from their salmon diet. How well these isolates survive in the marine environment is also not known since in general E. coli are not salt tolerant.

The current study on MRSA isolated from Nepal suggests that humans are the source of the MRSA identified both in the macaques and the environment and may be linked to humans feeding the primates. The Neplaese MRSA strains (CC22, CC361, CC772) indicate epidemiological links to other countries within the Indian subcontinent and to the Middle East. Strains that have been detected in monkeys have been found in humans, either in this or in other studies. For the environmental CC779-MRSA-VT, no conclusions can be drawn due to a lack of data. However, this lineage has been found in humans before (see above) and we are not aware of any published observations on its presence in animals. In conclusion, it can be speculated that the detection of MRSA in Nepalese Rhesus can be attributed at least in a majority of cases to contamination/infection during contacts with humans or to human offal. Thus, humans can not only be infected with zoonotic pathogens by close contact with wild animals; they also might transmit human pathogens into wildlife, posing a possible hazard to wild animals whose population are already endangered and under stress. However, the impact on the monkeys might be limited in this particular case as a related species of macaques seemed to be rather resistant towards PVL [3].

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Submission declaration

Part of the ExPEC work has been previously published [10] but the current technical report provides new information that was not published in the short note and is used to contrast with MRSA work that has recently been published [11].

References

[1] D. Dolejska, I. Literak. Antimicrob Agents Chemoth. 63, (2019) e01167-19

[2] T. S. Evans, P. A. Barry, K. V. Gilardi, T. Goldstein, J. D. Deere, J. Fike, J. Yee, B. J. Ssebide, D. Karmacharya, M. R. Cranfield, D. Wolking, B. Smith, J. A. Mazet, C. K. Johnson. PLoS Neglected Tropical Dis. 9, (2015) :e0003813.

[3] B. Loffler, M. Hssain, M. Grundmeier, M. Brück, D. Holzinger, G. Varga, J. Roth, B. C. Kahl, R. A. Proctor, G. Peters. PloS Patho. 6, (2019), e1000715

[4] J. Lundin, R. Dills, G. Ylitalo, M. B. Hanson, C. K. Emmons, G. S. Schorr, J. Ahmad, J. A. Hempelmann, K. M. Parsons, S. K. Wasser. Arch Environ Contam Tox. 70 (2016) 9-19.

[5] D. Melendez, M. C. Roberts, A. L. Greninger, S. Weissman, D. No, P. M. Rabinowitz, S. Wasser. J. Antimicrob. Chemother. 74 (2019) 2176-2180

[6] K. E. Michael, D. No, M. C. Roberts. J Appl Microbiol 121 (2016) 846-854.

[7] S. Monecke, L. Jatzwauk, E. Müller, H. Nitschke, K. Pfohl, P. Slickers, A. Reissig, A. Ruppelt-Lorz, R. Ehricht. PLoS One 11 (2016) e0162654.

[8] S. Monecke, G. Coombs, A. C. Shore, D. C. Coleman, P. Akpaka, M. Borg, H. Chow, M. Ip , L. Jatzwauk, D. Jonas, K. Kadlec, A. Kearns, F. Laurent, F. G. O'Brien, J. Pearson, A. Ruppelt, S. Schwarz, E. Scicluna, P. Slickers, H. L. Tan, S. Weber, R. Ehricht. PloS One 6 (2011) e17936.

[9] Port J.A., Wallace J.C., Griffith W.C., Faustman E.M. PloS One 10 (2012) e4800.

[10] M. C. Roberts, P. R. Joshi, A. L. Greninger, D. Melendez, S. Paudel, M. Acharya, N. K. Bimali, N. P. Koju, D. No, M. Chalise, R. C. Kyes. FEMS Microbiol Eco.1 5 (2018) 94.

[11] M. C. Roberts, P. R. Joshi, S. Monecke, R. Ehricht, E. Muller, D. Gawlik, S. Paudel, M. Acharya, S. Bhattarai, S. Pokharel, R. Tuladhar, M. K. Chalise, R. C. Kyes. Frontier in Microb. (2019)

SPECIAL ISSUE

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Differential expression of multidrug-resistance genes in *Trichophyton rubrum*

Maíra Pompeu Martins, Antonio Rossi*, Pablo Rodrigo Sanches, Nilce Maria Martinez-Rossi

Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, 14049-900, Ribeirão Preto, SP, Brazil

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ABSTRACT

Treatment of dermatophytosis is generally a long and challenging process, deeply affected by drug resistance owing to efflux-mediated activity. These drug-pumping mechanisms involve overexpression of transporter proteins with the ability to extrude a wide variety of structurally and functionally unrelated compounds. The ATP-binding cassette transporter and the major facilitator are the two largest superfamilies of transporters, expressed ubiquitously in all living organisms. Here, we examined the transcription modulation of both families of transporter genes in the dermatophyte *Trichophyton rubrum* upon challenge with sub-lethal doses of undecanoic acid or acriflavine. Data derived from RNA sequencing revealed transporters functioning in specific patterns according to the stressing condition, suggesting that each drug recruits specific physiological pathways. Synergistic transport activity may be acting to overcome drug toxicity, demonstrating that multidrug resistance transporters cooperate to induce drug resistance and fungal survival in an unpredictable manner.

Keywords: RNA-seq; ATP-binding cassette superfamily; Major facilitator superfamily; Dermatophyte; Antifungal efflux

1. Introduction

Dermatophytes are a specialized group of filamentous fungi that colonize keratinized tissues. They are the most commonly diagnosed pathogens in superficial infections, with *Trichophyton rubrum* being responsible for the majority of infective cases [1, 2]. Treatment of dermatophytosis is generally a long and challenging process, deeply affected by the small number of available antifungal drugs, the limited number of cellular targets, and the occurrence of drug resistance [3, 4].

Among mechanisms that render the fungus resistant or tolerant to toxic compounds, overexpression of drug efflux pumps belonging to the ATP-binding cassette (ABC) superfamily or to the major facilitator superfamily (MFS) comprise a major challenge [5, 6]. Both superfamilies consist of integral membrane proteins, with a conserved domain architecture [5]. These multidrug resistance (MDR) transporter genes are active against diverse unrelated chemical compounds and extrude them from the cell [7, 8].

The large number of genes encoding these transporters and the clinical relevance of efflux-mediated drug resistance supports the need to elucidate the molecular features involved in transporter interactions and pumping activity [5]. Further, because of their association with the prominent efflux-mediated pleiotropic resistance, the relevance of these transporters in fungal pathogenicity that acts as a virulence factor is thus becoming evident [6, 9].

In dermatophytes, evaluation of transcription profiles of ABC transporter genes *pdr1, mdr2,* and *mdr4* showed a synergistic activity among them in response to antifungal drug exposure. Further, among the four dermatophytes evaluated, each presented a gene-specific transcriptional profile [10]. The Δ*mdr2* mutant strain showed reduced infectivity on human nails and an enhanced sensitivity to drugs including terbinafine [6, 11]. The transcript levels of the *mdr1* gene were observed to be induced in response to drugs such as griseofulvin and itraconazole, suggesting its participation in antifungal resistance [12]. These results indicate a particular efflux activity, dependent on the chosen

***Corresponding author:** Antonio Rossi, anrossi@usp.br

drug, the gene evaluated, and the organism analyzed.

Global gene-expression analysis is a prominent approach to evaluate modulatory changes during environmental challenge. Thus, through RNA sequencing (RNA-seq) interpretation of a myriad molecular functions in diverse organisms has been favored [13, 14]. In *Trichophyton rubrum*, two RNA-seq data revealed transcriptional modulation in response to antifungal-active compounds, undecanoic acid and acriflavine [15, 16]. Among the identified differentially expressed genes, we selected those coding for ABC and MFS transporters and evaluated their transcriptional profile in response to stressing conditions.

Here we aimed to evaluate the drug efflux-related gene expression, attempting to examine the role of these drugs in modulating gene expression.

2. Materials and Methods

2.1. Data analysis and gene selection

The prediction of ABC and MFS domain-containing proteins was performed using the HMMER v3.1b2 pipeline [17] in the *T. rubrum* CBS 118892 (Centraalbureau voor Schimmelcultures, The Netherlands) genome sequence, available at ftp://ftp.broadinstitute.org/pub/annotation/fungi . The hidden Markov model (HMM) was built utilizing a Pfam multiple alignment based-search of 55 sequences corresponding to the ABC domain-containing proteins and other Pfam multiple alignment based-search of 192 sequences corresponding to the MFS domain-containing proteins, both from different organisms (https://pfam.xfam.org/family/PF00005 and https://pfam.xfam.org/family/PF07690 respectively). The resulting models with epitopes of consensus sequences of ABC and MFS domain-containing proteins were used to search for homologs in *T. rubrum* protein sequences. The genes, whose codes for the hereinafter identified proteins were confronted with the differentially expressed genes (DEG) identified in the *T. rubrum* undecanoic acid and acriflavine RNA-seq libraries, are available at the GEO database under accession nos. GSE102872 and GSE40425. The selected genes are presented in Table 1.

2.2. T. rubrum strain and culture conditions used in the RNA -seq libraries assemblage

T. rubrum mycelia obtained from 96 h-culture, starting from approximately 10^6 conidia mL^{-1} in Sabouraud dextrose broth (SDB), challenged with 1.75 µg/mL of acriflavine (Sigma Aldrich Corp., USA), which corresponds to 70% of its MIC (minimal inhibitory concentration), or with 17.5 µg mL-1 (70% MIC) of undecanoic acid (Sigma Aldrich Corp., USA) were used for RNA extraction and sequencing as previously published [15, 16]. The strain was maintained as described previously [18, 19].

3. Results and discussion

A total of 44 DEGs coding for ABC or MFS transporters were identified in two libraries. Among them, 11 are responsive to both conditions (Table 1.A). Other 19 were modulated exclusively in response to acriflavine (Table 1.B), and 14 respond only to undecanoic acid exposure (Table 1.C). A higher number of MFS transporters were modulated in our experimental conditions, in relation to the ABC transporter genes.

Among the genes modulated in response to both tested drugs, four, identified as TERG_01443, TERG_08336, TERG_02283, and TERG_01623, were inversely modulated in response to the drugs chosen. Drug exposure repressed the other concomitantly modulated MDR transporter genes, four of them belonging to the ABC transporter family and the other three were MFS transporters. Two of the repressed MFSs were siderophore iron transporters.

In response to acriflavine, more genes were induced than repressed, belonging mainly to the MFS transporter superfamily than to the ABC transporters. Among the induced genes, we identified a phosphate permease that was highly modulated (log_2 = 9.58) presenting two MFS transporter domains.

Undecanoic acid modulated less MDR transporter genes than acriflavine, mainly inducing their expression rather than repressing it. As observed with acriflavine, more MFS transporter genes were responsive to undecanoic acid exposure. The inductive effect of MDR transporters is expressively more related to the initial exposure than to the later time period of exposure.

The smallest number of genes modulated in both conditions compared to those that are drug-specific highlights a drug-dependent activation. Under undecanoic acid challenge, these genes responded in the earliest time period. Except for the ABC transporter TERG_01443, the other three genes responded exclusively in the latest time period of exposure to acriflavine.

Only three of the DEGs were modulated at all time points, for each drug tested: TERG_06679, TERG_00955, and TERG_00402 remained repressed during the 24 h of acriflavine exposure, and TERG_08130, TERG_05055, and TERG_03719 remained downregulated in the two time points of exposure to undecanoic acid.

4.Discussion

Antifungal-active compounds challenge fungal survival. Through activation of efflux pumps such as the ATP-binding cassette (ABC) and the major facilitator superfamily (MFS) transporters, multiple cytotoxic chemicals are actively extruded from fungal cells, thus playing an important role in the multiresistance phenomenon [20]. These hostile environments provoked by each different substance force fungi to modulate diverse and specific transporters, seeking to counter their harmful effects.

Table 1 | MFS and ABC transporter genes modulated in response to acriflavine (ACF) or undecanoic acid (UDA) exposure in *T. rubrum*. Modulatory data correspond to the control (in the absence of drug) compared at each time point of drug exposure.

ID	ACF vs. control - log_2 (RNA-seq fold change)			UDA vs. control - log_2 (RNA-seq fold change)		Number of MFS or ABC Transporter* domains	HMM (E-value)	Gene Product Name
	3 h	12 h	24 h	3 h	12 h			
Table 1.A – modulated in response to exposure of ACF and UDA								
TERG_01443	1.80		1.52	-1.80		2*	4.10E-49	ABC multidrug transporter (*T. tonsurans*)
TERG_08336			-1.59	2.51		2	1.20E-45	MFS multidrug transporter, putative (*A. benhamiae*)
TERG_02283			-1.61	2.11		1	1.70E-32	MFS transporter, putative (*T. verrucosum*)
TERG_01623			1.72	-2.02		1	2.30E-39	MFS transporter (T. equinum)
TERG_08613		-1.70	-2.14		-2.04	2*	4.40E-72	ABC multidrug transporter mdr2, putative (*A. benhamiae*)
TERG_08130		-1.70	-1.77	-2.03	-2.28	2*	6.00E-36	ABC ATPase (*T. equinum*)
TERG_04323		-1.60	-1.56	-1.61		2*	1.00E-57	ATP-dependent bile acid permease (*T. equinum*)
TERG_05617	-2.04				-1.89	1*	3.00E-02	Hypothetical protein
TERG_06679	-2.67	-3.26	-3.82		-2.14	2	4.40E-18	MFS transporter, putative (*A. benhamiae*)
TERG_08619	-2.76	.	-1.72	-3.56		2	3.20E-17	Siderophore iron transporter mirB (*T. equinum*)
TERG_08620	-2.25	.	-2.61	-2.35		2	3.30E-18	Siderophore iron transporter (*T. equinum*)
Table 1.B – modulated only in response to exposure of ACF								
TERG_04952	2.14					1*	1.20E-35	Multidrug resistance protein (*T. equinum*)
TERG_07801		2.60	3.13			1*	6.64E-12	ABC multidrug transporter *mdr4*
TERG_00762	1.55		1.58			2*	9.60E-04	Vesicular-fusion protein SEC18 (*M. gypseum*)
TERG_07921	1.86					1*	3.90E-02	Denylylsulfate kinase
TERG_02583			9.58			2	1.30E-22	Phosphate permease (*A. benhamiae*)
TERG_04400	1.56					2	2.00E-21	MFS monosaccharide transporter, putative (*T. verrucosum*)
TERG_03174		3.69	4.97			2	7.50E-11	MFS siderochrome iron transporter MirB (*T. verrucosum*)
TERG_02369		1.81				1	5.50E-36	MFS transporter (*T. tonsurans*)
TERG_08059		1.70					1.40E-18	Sugar transporter (*T. equinum*)
TERG_01623			1.72			1	4.16E-12	MFS transporter
TERG_03933			-1.73			2*	2.00E-53	ABC metal ion transporter
TERG_00955	-1.66	-1.79	-2.29			2*	1.30E-52	ABC drug exporter AtrF (*T. verrucosum*)
TERG_00402	-1.60	-2.10	-2.25			1*	2.30E-19	ABC multidrug transporter, putative (*T. verrucosum*)
TERG_07216	-1.50					1*	1.10E-01	Hypothetical protein
TERG_00008			-1.68			2	6.70E-19	MFS phospholipid transporter (*T. tonsurans*)
TERG_00820			-2.30			2	7.90E-47	MFS multidrug transporter, putative (*A. benhamiae*)
TERG_05153		-1.89	-2.37			2	3.50E-26	MFS transporter, putative (*A. benhamiae*)
TERG_07539	-1.64		-3.49			1	6.20E-48	Multidrug resistance protein (*T. tonsurans*)
TERG_06650	-1.65					2	2.70E-30	MFS monocarboxylate transporter, putative (*A. benhamiae*)
Table 1.C – modulated only in response to exposure of UDA								
TERG_04224				2.41		2*	7.70E-55	ABC transporter
TERG_02508				1.91		2*	1.40E-44	ABC multidrug transporter, putative (*A. benhamiae*)
TERG_06361				2.13		1*	1.60E-02	ATP-dependent protease La
TERG_00162				3.88		1	1.80E-47	MFS multidrug transporter, putative (*A. benhamiae*)
TERG_00163				2.12		2	7.40E-19	Siderochrome-iron transporter, putative (*A. benhamiae*)
TERG_05575				2.65		2	7.70E-42	MFS multidrug transporter (*T. tonsurans*)
TERG_05199				2.06		2	1.30E-40	MFS gliotoxin efflux transporter GliA (*T. verrucosum*)
TERG_05466					1.80	2	6.80E-25	MFS transporter, putative (*T. verrucosum*)
TERG_04227				-2.60		2*	4.40E-38	ABC transporter (*T. tonsurans*)
TERG_04514				-1.86		1*	8.30E-02	Cell division control protein 12 (*T. tonsurans*)
TERG_04308				-2.54		2	1.40E-30	MFS sugar transporter (*T. tonsurans*)
TERG_03984					-1.86	1	3.90E-37	Major facilitator superfamily transporter MFS-1 (*M. canis*)
TERG_05055				-2.02	-1.64	1	4.80E-44	MFS multidrug transporter (*T. tonsurans*)
TERG_03719				-2.84	-2.87	2	4.60E-11	MFS sugar transporter (*T. tonsurans*)

We examined the transcription modulation of ABC and MFS transporter genes in the dermatophyte *T. rubrum* challenged with sub-lethal doses of undecanoic acid or acriflavine. The differentially expressed genes were subdivided in three groups: those responsive to both drugs, and genes transcribed exclusively in response to each of the chosen drugs.

A more elevated number of DEGs belonging to the MFS superfamily, comparing to the ABC transporter genes, were identified in both libraries, including three genes commonly expressed and inversely modulated in the presence of the evaluated drugs. The MFS transporters correspond to the largest class of secondary active pumps in all branches of life, and the high number of gene copies indicates a highly conserved defense potential [20, 21]. MFS are capable of transporting a huge variety of substances, ranging from small solutes, in response to chemiosmotic ion gradients, to drugs presumably acting as Drug:H+ Antiporters (DHA) [20, 22].

Here, important physiological functions are affected by the drugs including the transport of siderophore-iron chelates (TERG_08619, TERG_08620, and others). As previously proposed, *T. rubrum* requires iron to overcome toxicity triggered by acriflavine exposure [15]. Since undecanoic acid also induced siderophore-related genes we supposed that an essential modulation profile of iron-related genes plays a role in stress resistance. Also affected, one sugar transporter is induced in response to undecanoic acid (TERG_08059), and the other two are repressed in the presence of acriflavine (TERG_04308, and TERG_03719). Sugar transport occurs along a concentration gradient, or operates when the availability of sugars presents relatively low concentrations [23]. Resistance to acriflavine and ethidium bromide was attributed to the *qacA* transporter from *Staphylococcus aureus*, a sugar uptake-related protein [24]. The evaluated drugs seem to oppositely affect the sugar availability in cells, recruiting MFS transporters in a particular way.

Among the induced MFS transporters, one phosphate permease is expressively upmodulated in response to acriflavine. In the presence of inorganic phosphate, Escherichia coli strains become more sensitive to acriflavine, despite the resistance they present in its absence [25]. Also, the yeast *Hansenula jadinii*, when challenged with increasing amounts of acriflavine, augments phosphorylation activity, relating the toxic effects of the drug to the phosphate availability on cells [26].

The DEGs identified as modulated in both drug conditions, excluding the inversely transcribed ones, are all repressed (Table 1.A). These genes are supposed to be more directly related to the drug extrusion activity. As they are modulated in all time points, their active recruitment appears to be time- and drug-dependent. Among those genes, the ABC ATPase (TERG_08130) is repressed in response to the two time periods of exposure to undecanoic acid. It is related to the molybdenum cofactor biosynthesis protein of *Talaromyces marneffei* thus, being related to cofactors or prosthetic group transport. Undecanoic acid also represses other cofactor-related exchangers including iron and copper transporters, suggesting an interconnection between the harmful effect of the drug to essential cycles such as carbon, sulfur, and nitrogen [27]. The putative MFS transporter (TERG_06679), repressed in response to acriflavine at all time points, is correlated to the protein phosphatase 2C from *Aspergillus oryzae* and may be repressed in an attempt to counterbalance the activity of other active phosphatases.

The transcription of the *mdr2* gene (TERG_08613), repressed in response to both drugs, is also presumably related to drug resistance. This ABC transporter and the *mdr4* gene (TERG_07801), responsive to acriflavine exposure, were previously evaluated in four dermatophyte fungi, including the herein evaluated *T. rubrum* [10]. Disruption of the *mdr2* gene induced high transcription levels of *mdr4* in the presence of griseofulvin, suggesting a counter activity of the *mdr4* gene overlapping the *mdr2* inactivation, thus providing resistance to this antifungal. With acriflavine, we observed an inverse pattern of *mdr2* and *mdr4* modulation indicating activation of *mdr4* in response to the repression of *mdr2* [15].

In different situations, it is possible to identify a synergism of activity among transporters that are apparently redundant in number and potential activity and are active in improving stress tolerance and surpassing physiological challenges in a drug-specific manner. The concomitant modulation of several MDR transporters highlights their biological importance and suggests an active bias to stimulate drug resistance, concurring somehow with fungal defense.

5.Concluding Remarks:

Our results suggest a drug-specific activation of efflux pumps, resulting in a particular pattern of transcriptional regulation, possibly, resulting in a drug-specific profiling of antifungal drug resistance. We also suggest a synergistic activity of these transporters, with a compensatory activity against stressing conditions. These observations point to a singular fungal-response that supports how antifungal drug resistance varies drastically among organisms and drug classes.

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Assistance - FAEPA of the HCFMRP-USP.

Refrences

[1] G.F. Persinoti, D.A. Martinez, W. Li, A. Dogen, R.B. Billmyre, A. Averette, J.M. Goldberg, T. Shea, S. Young, Q. Zeng, B.G. Oliver, R. Barton, B. Metin, S. Hilmioglu-Polat, M. Ilkit, Y. Graser, N.M. Martinez-Rossi, T.C. White, J. Heitman, C.A. Cuomo, Genetics, 208 (2018) 1657-1669.

[2] P. Zhan, W. Liu, Mycopathologia, 182 (2017) 77-86.

[3] N.M. Martinez-Rossi, N.T. Peres, A. Rossi, Mycopathologia, 166 (2008) 369-383.

[4] T.R. Jacob, N.T. Peres, M.P. Martins, E.A. Lang, P.R. Sanches, A. Rossi, N.M. Martinez-Rossi, Front Microbiol, 6 (2015) 1241.

[5] R. Prasad, M.K. Rawal, Front Pharmacol, 5 (2014) 202.

[6] F.C. Maranhão, F.G. Paião, A.L. Fachin, N.M. Martinez-Rossi, J Med Microbiol, 58 (2009) 163-168.

[7] L.E. Cowen, D. Sanglard, S.J. Howard, P.D. Rogers, D.S. Perlin, Cold Spring Harb Perspect Med, 5 (2015) a019752.

[8] N.M. Martinez-Rossi, T.A. Bitencourt, N.T.A. Peres, E.A.S. Lang, E.V. Gomes, N.R. Quaresemin, M.P. Martins, L. Lopes, A. Rossi, Front Microbiol, 9 (2018) 1108.

[9] J.J. Coleman, E. Mylonakis, PLoS Pathog, 5 (2009) e1000486.

[10] M.P. Martins, A.C. Franceschini, T.R. Jacob, A. Rossi, N.M. Martinez-Rossi, J Med Microbiol, 65 (2016) 605-610.

[11] A.L. Fachin, M.S. Ferreira-Nozawa, W. Maccheroni, Jr., N.M. Martinez-Rossi, J Med Microbiol, 55 (2006) 1093-1099.

[12] E.P. Cervelatti, A.L. Fachin, M.S. Ferreira-Nozawa, N.M. Martinez-Rossi, Med Mycol, 44 (2006) 141-147.

[13] Z. Gao, Z. Zhao, W. Tang, Front Genet, 9 (2018) 588.

[14] K.R. Kukurba, S.B. Montgomery, Cold Spring Harb Protoc, 2015 (2015) 951-969.

[15] G. Persinoti, N. de Aguiar Peres, T. Jacob, A. Rossi, R. Vencio, N. Martinez-Rossi, BMC Genomics, 15 (2014) S1.

[16] N.S. Mendes, T.A. Bitencourt, P.R. Sanches, R. Silva-Rocha, N.M. Martinez-Rossi, A. Rossi, Sci Rep, 8 (2018) 2520.

[17] R.D. Finn, J. Clements, S.R. Eddy, Nucleic Acids Res, 39 (2011) W29-37.

[18] A.L. Fachin, C.M. Maffei, N.M. Martinez-Rossi, Mycopathologia, 135 (1996) 141-143.

[19] T.R. Jacob, N.T. Peres, G.F. Persinoti, L.G. Silva, M. Mazucato, A. Rossi, N.M. Martinez-Rossi, Med Mycol, 50 (2012) 368-377.

[20] S.C. dos Santos, M.C. Teixeira, P.J. Dias, I. Sa-Correia, Front Physiol, 5 (2014) 180.

[21] X.C. Zhang, Y. Zhao, J. Heng, D. Jiang, Protein Sci, 24 (2015) 1560-1579.

[22] E.M. Quistgaard, C. Low, F. Guettou, P. Nordlund, Nat Rev Mol Cell Biol, 17 (2016) 123-132. DOI: 10.1038/nrm.2015.25

[23] M.J. Leandro, C. Fonseca, P. Goncalves, FEMS Yeast Res, 9 (2009) 511-525.

[24] D.A. Rouch, D.S. Cram, D. DiBerardino, T.G. Littlejohn, R.A. Skurray, Mol Microbiol, 4 (1990) 2051-2062.

[25] H. Nakamura, A. Suganuma, J. Greenberg, J Gen Microbiol, 91 (1975) 45-52.

[26] A. Kimura, Y. Tatsutomi, H. Fukuda, H. Morioka, Biochim Biophys Acta, 629 (1980) 217-224.

[27] D. Kaufholdt, C.K. Baillie, R. Meinen, R.R. Mendel, R. Hansch, Front Plant Sci, 8 (2017) 1946. DOI: 10.3389/fpls.2017.01946 Hansch, Front Plant Sci, 8 (2017) 1946.

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A Model of How Antibiotics Work

Eva M. Ogens

Ramapo College of New Jersey, 505 Ramapo Valley Road, Mahmah, NJ 07430, USA

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ABSTRACT

Almost all children have taken antibiotics as a result of ear infections, strep throat, or other bacterial infections. Some of them feel better soon and don't understand why they have to keep taking the medication for the full ten days as prescribed. Others forget to take the medicine, and then often have to be put on a stronger type of antibiotics. This game enables students to experience a model of the effects of antibiotics on a population of disease-causing bacteria during an infection. Students learn how variables such as skipping a day of medication affect the persistence of the disease. A key concept is that almost every naturally occurring population of bacteria that cause disease has a component that is resistant to antibiotics. By graphing data, students can visually understand why it is important to take a complete course of antibiotics to kill all the bacteria and decease the likelihood of bacteria becoming resistant, which can be harmful to human health and is a major public health problem.

Keywords: Antibiotic resistance, Antibiotic education, Model, Simulation game

1. Introduction

As a Professor of Education, and not a research scientist, the 3rd International Caparica Conference on Antibiotic Resistance (IC2AR) opened my eyes to the widespread and serious problem of antibiotic resistance which continues to be a global public health issue. Dr. Jose Capelo[1], in his welcome speech and introduction in the book of abstracts, stated the problem has escalated to levels where the need to "require medical assistance in a hospital has become a Russian roulette, as such is the risk to get infected with a bacteria resistant to antibiotics." This statement rang so true when a friend of mine, who recently had surgery, had a raging infection in his incision. It has been estimated that by 2050, 10 million lives per year will be at risk from antibiotic-resistant infections [2]. Professor Jose Capelo (2019) stated we are all "soldiers in the battle of antibiotic resistance." [1].

Because antibiotics still provide highly effective treatments for common diseases with important implications for human health, their proper use is essential. The challenge for public education is to achieve a meaningful reduction in unnecessary antibiotic use without adversely affecting the management of bacterial infections [3]. Antibiotics are the most commonly prescribed therapy among all medications given to children [4].

"Children have the highest rates of antibiotic use and they also have the highest rate of infections caused by antibiotic-resistant pathogens, but antibiotics are not necessary for the majority of infections seen in the pediatrician's office. Parent pressure can influence a doctor's decision about using antibiotics. Doctors prescribe antibiotics much more often for children if they think parents expect them, but less often if they feel parents do not expect them" [5].

By using a model of how antibiotics work which is actually a game, students can learn why it is important to take antibiotics as prescribed. Using a partial dose or stopping because they feel better leads to antibiotic resistance. As stated in the Stanford medical health bulletin to parents:

"When an illness does require antibiotic treatment, it's important that your child take the medication exactly as prescribed by your pediatrician. Don't stop having your child take the medication because he or she starts to feel better. Just as overuse of antibiotics leads to resistant bacteria, so does using only a partial dose. Each time

***Corresponding author:** Eva M. Ogens, Ed.D. 1,1.Ramapo College of New Jersey, 505 Ramapo Valley Road, Mahwah, NJ 07430 USA. Email address: eogens@ramapo.edu +1 973 632-3969

antibiotics are taken, sensitive bacteria are killed, but resistant ones may be left to grow and multiply, according to the CDC" [5].

The objective of this article is to describe the use of a simple model to educate young people and their parents, in fact any person who is not scientifically knowledgeable, on why antibiotics should be taken as prescribed to avoid the development of antibiotic resistance.

2. Materials and Methods

2.1. Background

Have you ever taken antibiotics? Imagine you are sick with a bacterial infection. Your doctor prescribes an antibiotic, normally taken for 10 days. Did you follow the directions completely? All antibiotics need to be taken as directed, which usually means taking all the pills and not stopping even if you begin feeling better. Why? When harmful bacteria appear on the scene, your body's immune system can usually keep a small population of them under control. If, however, these bacteria reproduce too quickly, you suffer the consequences of an infection. Antibiotics help your body fight off an infection by killing these harmful bacteria. Unfortunately, a small number of bacteria in any population may not be affected by the antibiotic as quickly. These bacteria, which are considered more resistant to the treatment, continue to reproduce and grow. Completing the full course of the antibiotic as prescribed by your doctor helps to make sure that these bacteria do not survive and therefore won't make you ill or infect someone else. This game is a model of how it works.

2.2. Materials needed

Colored disks- any three colors, available on Amazon (transparent colored counting chips), playing dice – one for each pair of players. In this activity, we used green or yellow, blue or purple, and orange or red.

Colored disks represent the bacteria in your body: Green (or yellow) disks represent the least resistant bacteria, blue (or purple) represent the resistant bacteria, and orange (red) represents the most resistant bacteria.

2.3. Materials needed

1. In this activity you will work with your partner to collect data. Begin with 20 disks, 13 green, 6 blue, and 1 orange. These disks represent the harmful bacteria living in your body before you begin to take the antibiotic. Set the extra disks aside for now.

2. It is time to take your antibiotic. Toss the die and follow the directions on Table 1.

3. Record the number of each type of bacteria in your body in Table 2.

4. Repeat #2 until all bacteria have been killed!

Table 1 | Game Instructions

You Toss	What Happened	What to Do	Notes:
1,3,5,6	You took the antibiotic on time, so bacteria are being killed!	Remove 5 disks, starting with the green disks first, since they are least resistant, then the blue, and last the orange.	The bacteria are reproducing all of the time! As long as any disks of any color remain, each time add one disk of each color to show they are still multiplying! For example, if you have resistant (blue) and extremely resistant (orange) bacteria in your body, add 1 blue disk and 1 orange disk to your
2,4	You forgot to take the antibiotic.	If there are any disks of any color remaining, add one disk of each color remaining to represent the fact that they are still multiplying!	

Table 2 | Chart for recording number of harmful bacteria in your body [6] (SEPUP, 2010, C-269).

Round Number	Least resistant Bacteria	Resistant Bacteria	Extremely Resistant Bacteria	Total
Initial	13	6	1	20
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

5. Use the data to graph the population for each type of bacteria and for the total number of bacteria on the graph paper provided. In this example (Supplementary material, Table S1), the bacteria took 12 days to die completely because the antibiotic was forgotten three times.

After the data is collected, it should be graphed to show how the least resistant bacteria die first, followed by the next resistant bacteria, and finally, the most resistant bacteria. The graph should visually show, if the antibitoic was forgotten for a day or two, how the bacteria immediately reproduce (Supplementary Material, Figure S1).

3. Results and discussion

To assess the value of this presentation, pre (dispositions) and post (perceptions) surveys were conducted. Fifteen respondents answered the following questions, using a Likert scale from strongly agree (5), agree (4), neutral (3),

disagree (2), strongly disagree (1).
Pre survey:
1. I use modeling in my own work.
2. I was aware of using games to model antibiotic resistance prior to this session.
3. I believe models are an effective tool for the training and education of future scientists.
4. Most people without a science background understand how antibiotics work.
Post survey:
1. This particular game appears to be an effective illustration for people to gain an understanding of how antibiotics work.
2. The presentation enhanced my belief in the usefulness of modeling in engaging the interest and understanding of scientific thinking.
3. The presentation contained practical examples and useful techniques that applied to current work.
4. The presentation made me think of things in a new way or see a different perspective.
5. The presentation had a lot of good information and ideas.
6. I was aware of this type of model prior to this session.
7. The presentation was concise and informative.
8. The presenter answered questions effectively.
9. The presenter maintained my interest during the entire presentation.
10. The presenter was knowledgeable about the topic and any related issues.
11. The presentation got people involved and interacting in a comfortable environment.
12. The presentation should be offered again.

4.Discussion

Overall, the presentation was well-received and perceptions of the use of model(s) were improved by this presentation.

The averages of the Pre survey are showed in Supplementary Material Table S2 and the averages of the post survey are showed in Supplementary Material Table S3.

In addition, when the activity was originally done with sixth grade students [7], the following feedback was received:

"I learned that even if you take antibiotics, the bs acteria keep multiplying. That is why you must take antibioticfor an extended period of time."

"I liked this project because it helped me understand how an infection works; if you don't treat it the bacteria get stronger."

"I learned that bacteria grow in a certain way. I thought it was cool and I had a lot of fun on learning about bacteria."

"I liked the activity. Something I learned is [that] the bacteria still grow when you are getting better."

"It showed me how to take care of myself better."

According to the Next Generation Science Standards, adopted by over 20 states in the United States, and representing about 36% of all students, modeling is one of the practices used by scientists and engineers. Students are encouraged to use models as a helpful tool for representing ideas and explanations. This model is a fun way for students and others to interactively see and understand what happens when antibiotics are misused.

Even those with a great deal of scientific and medical knowledge recognized the value of modeling antibiotic resistance. After the presentation at the IC2AR, one immunologist commented, "The model surprised me when I drew the graph! (It) made me realize how (a) simple model can help understanding of a complex issue." Another participant said, "You provided me a new form to teach to my students the ARM phenomenon."

5.Concluding Remarks:

One of the major contributors to the inappropriate use of antibiotics is based on insufficient knowledge and therefore education about prudent antibiotic use aimed at both the prescribers and the public is important [8]. Educating young people by using this model of antibiotic resistance may be one key to meeting the challenges of the battle against antibiotic resistance. If the young people and their parents are more knowledgeable, perhaps parents would not put pressure on doctors to prescribe antibiotics when not really needed, and each generation will become more savvy about the proper use of these valuable drugs to combat infections.

Perhaps we are fighting a losing battle as bacteria continue to evolve. But we can make some progress by educating the public about this global problem. Malala Yousafzai said it eloquently, "There are many problems, but I think there is a solution to all these problems; it's just one, and it's education."

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Refrences

[1] José Luis Capelo, Introductory Welcome Speech, 3rd International Caparica Conference on Antibiotic Resistance, June 10, 2019
[2] Ryan O'Hare (February 6, 2018) https://medicalxpress.com/news/2018-02-children-drug-resistant-bugs-common-antibiotics.html).
[3] H. Goossens, D. Guillemot, M. Ferech, B. Schlemmer, M. Costers, M. van Breda, L. J. Baker, P. G. Davey, O. Cars,. (2006) https://www.ncbi.nlm.nih.gov/pubmed/16568344
[4] Elizabeth T. Rogowski, James A. Platt-Mills, Jessica C. Seidman, John Sushil, Mustafa Mahfuz, Ulak Manjeswori,

Sanjaya K. Shrestha, Sajid Bashir Soofi, Pablo Penataro et al. Bulletin World Health Organization. 95 (2017) 1 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5180352/

[5] Curb antibiotic abuse in children https://www.stanfordchildrens.org/en/topic/default?id=curb-antibiotic-abuse-in-children-1-2165

[6] Science for Public Understanding Program (SEPUP) Activity 51, The Full Course, from Issues and Life Science (2012), the Lawrence Hall of Science, university of California, Berkeley, CA. Published by Lab-Aids, Ronkonkoma, NY

[7] Eva M. Ogens, Richard Langheim. The American Biology Teacher, 78 (2016) 7.

[8] Chang-Ro Lee, Jung Hun Lee, Lin-Woo Kang, Byeong Chul Jeong, Sang Hee Lee. Biomedical Research InternationaL (2015),

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NIMA-related kinase 7 interacts with Mat1 and is involved in the UV-induced DNA Damage Response

Arina Marina Perez[1], Edmarcia Elisa de Souza[1], Jörg Kobarg[1,2*]

[1] Programa de Pós-graduação em Biologia Funcional e Molecular, Departamento de Bioquímica e BiologiaTecidual, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, 13083-862 SP, Brazil; [2] Faculdade de Ciências Farmacêuticas, Universidade Estadual de Campinas, Campinas, 13083-859 SP, Brazil

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ABSTRACT

Nek7 is a serine/threonine kinase of the mammalian NIMA-related kinases (Neks) family, which members are involved in the regulation of the progression of the cell cycle. Although several Nek members have been associated with a range of cell cycle-related tasks, including DNA repair, a possible role of Nek7 in the DNA-damage response is so far unknown. Here, we employed in vitro and in vivo interaction assays to identify Mat1 as a specific Nek7 binding partner and substrate. In addition, we showed that Nek7 pulled down both CDK7 and cyclin H and directly phosphorylated Mat1, indicating that Nek7 may play a role in the regulation of the CAK complex. Furthermore, we showed that both Nek7 and Mat1 depletion led to an accumulation of cells in the S-phase, decreased cell proliferation and increased apoptosis. Notably, the mutational ablation of kinase Nek7 activity also induced increased apoptosis upon DNA damage. Collectively, our findings support the notion that Nek7 may cooperate with Mat1 in signal pathways that govern the cell cycle machinery including DDR, S-phase progression and apoptosis, and thereby can constitute an important novel player for in the context of cellular transformation and tumorigenesis.

Keywords: Nek7, Mat1, CAK complex, apoptosis, DNA damage response

1. Introduction

The human Nek7 belongs to the NIMA-related kinases or "Neks" family, that comprises a family of eleven proteins named Nek1 to Nek 11, which have been functionally associated to mitosis, cilium regulation and DNA damage response (DDR) [1].

Nek7 interacts with Nek6 and Nek9, both of which are implicated in cell cycle progression and spindle assembly [1-6]. Specifically, silencing of Nek7 causes alterations in levels of γ-tubulin in interphase cells and results in an arrest in prometaphase, whereas its over-expression results in multinucleated cells and a high proportion of apoptotic cells [4]. In addition, reduced interphase-microtubules growth and contraction speed were observed after Nek7 suppression, showing that Nek7 influences microtubule dynamics [6]. Salem and colleagues [7] showed that Nek7 absence is lethal during embryogenesis, indicating the importance of Nek7 in the development and survival of the organism. Moreover, additional studies have found higher Nek7 expression levels in cancers of larynx, breast, colorectal and gallbladder [8]. Recently, studies showed that NEK7 plays an important role in the regulation of NLRP3 inflammasome activation [9] and its overexpression induces the production of abnormal cells, including the multinucleated cells and apoptotic cells which are closely linked to inflammation [10]. Moreover, Nek7 might be involved in hepatocarcinoma progression by regulating cyclin B1 expression [11]." More recently, the recruitment of Nek7 was shown in TR1 regulation, in response to oxidative telomeric DNA damage [12], demonstrating once more the typical multi-functionality

***Corresponding author:** Jorg Kobarg, jorgkoba@unicamp.br; Tel.: +55-19-3521-1443.

found for many protein kinases. Thus, these findings suggest that Nek7 is an essential component to cell division regulation and consequently a possible involvement in tumorigenesis maybe predicted.

We have previously shown in yeast two-hybrid screens that Nek7 interacts with Cyclin-dependent kinase (CDK)-activating kinase assembly factor Mat1 [13], which along with cyclin H and CDK7 forms the CDK-activating kinase (CAK) complex [14]. CAK complex composes the kinase subunit of the basal Transcription factor IIH (TFIIH), that participates in nucleotide excision repair (NER) [15], transcriptional and cell cycle regulation [16]. Recently Patel and co-workers [17] found that expression of CDK7, Cyclin H and MAT1 is elevated in breast cancer, suggesting that this tumor type may be especially sensitive to CDK7 inhibition and that the CDK7 over-expression may contribute to an elevated chemo-resistance of cancer cells in comparison to normal tissues. In essence, Mat1 plays a role in the cell cycle control by modulating the expression of CDK7 and cyclin H [18], determining the CAK substrate specificity towards important cell cycle players such as p53 [19] and pRb [20], and monitoring the TFIIH-DNA damage response [21]. However, no record exists about upstream Mat1 players in regard to cell cycle regulation. In addition, although we previously speculated about a possible connection of Nek7 with the DDR, based on in silico analyses [1], no experimental data so far reported an involvement of Nek7 in the DDR.

Here, we provide the first evidence of functional involvement of Nek7 in the DDR. Through in vitro and in vivo interaction studies, we demonstrate that Mat1 is a novel Nek7 interactor and substrate. Our observations also indicate that Nek7 associates to the other CAK proteins CDK7 and Cyclin H, indicating that Nek7 could participate in the regulation of CAKs tasks. Since Mat1 is a member of the CAK kinase that is crucial for DNA repair and cell cycle progression, we investigated the Nek7 involvement along with Mat1 in DDR and cell cycle regulation. In this regard, we showed that Nek7 or Mat1 depletion led to an accumulation of cells in the S-phase and decreased cell proliferation. Importantly, we further found that expression of a kinase inactive variant of Nek7 leads to increased levels of apoptosis, upon UV-light induced DNA damage. Based on our results we propose that Nek7 and Mat1 are involved in monitoring the integrity of the genome and to protect cells from accumulating genetic damage.

2. Materials and Methods

2.1. Immunoprecipitation

Cells were lysed in lysis buffer [20 mM Hepes (pH 7.4), 150 mM NaCl, 1% Triton, 10% Glycerol, 1 mM EGTA, 1mM EDTA, protease and phosphatase inhibitor cocktail (1:100, SIGMA)]. Cell lysates were incubated and gently rocked for 1h, at 4 °C, in ANTI-FLAG® M2 Affinity gel. Resin and them the immune complexes were precipitated, washed using TBS1X and were subjected to immunoblot analysis.

2.2. Recombinant protein purification and Pull Down assay

Full-length Nek7 (6×His-Nek7) construct was obtained as previously reported by de Souza [13]. For pull down assay, 100 µl Ni-NTA Agarose (Qiagen), was washed twice with 1 mL of wash buffer (50 mM Tris-HCl, pH 8,0; 1% NP40 and 1% protease inhibitor). Then, 100 µg of 6×His-Nek7 was bound to the Ni-NTA agarose resin and incubated for four hours, by gentle rocking at 4°C. The resin containing the 6×His-Nek7 was washed three times with 1 mL of wash buffer, and incubated over-night at 4°C with 800 µg HEK293T lysate. Afterwards, the resin containing the protein-protein complex was washed three times with 1mL of wash buffer, and analyzed by SDS-PAGE and Western blotting, using the indicated specific antibodies against the tagged proteins.

2.3. In vitro kinase assay

For In vitro kinase assay, 0.5 µg of GST-Nek7 and 0.5 µg of each substrate were suspended in kinase assay buffer (20 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 1 mM NaF, 2 mM β-glycerophosphate, 100 µM ATP, 1 mM dithiothreitol), supplemented with 1 µCi of [γ-P_{32}]-ATP, and incubated at room temperature for 1 hour. SDS-PAGE sample buffer was then added, to stop the reaction, followed by incubation at 100°C for 5 minutes. Proteins were then separated by SDS-PAGE, and gel was dried and autoradiographed.

2.4. Cell culture and plasmid transfections

HEK293T and HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum. Cells were transfected using Lipofectamine reagent. 4 µg DNA was added to 700 µl of optiMEM and 30 µl of lipofectamine and this mixture was incubated for 20 minutes at room temperature. The mixture was subsequently added to the cell growth medium, and cells were incubated for 48 h prior to medium exchange.

2.5. Viral transduction

The Nek7 and Mat1 knock down lines were produced by viral transfection using a lentiviral system carrying short-interfering RNAs (shRNAs) designed to target human Nek7 or Mat1: - shRNANek7 = TRCN0000001967,5′-CTTTAGTTGGTACGCCTTATT-3′ (generating clone Nek7-N767), - shRNANek7 = TRCN0000001969, 5′-GAAGAGTGTAACCAAAGTAAT-3′ (generating clone Nek7-N769); - shRNAMat1= TRCN0000019944, 5′-CCTAGTCTAAGAGAATACAAT-3′ (generating clone

Mat1.1), - shRNAMat1 = TRCN0000019945, 5′-GCTATACTTCTTCTCTTGCTT-3′(generating clone Mat1.2), all purchased from The RNAi Consortium (TRC) (UMASSmed core, Worchester, USA). The lentiviral particles were introduced into HeLa cells according to the manufacturer's instructions and stable cell lines were generated by selection with 3.5 µg/ml puromycin (Sigma-Aldrich). Two stably transfected clones named N769, N767 (for Nek7) and two others for Mat1 (named MAT1.1 and MAT1.2) were obtained and analyzed. The efficacy of Nek7 and Mat1-depletion was assessed by Western Blotting (WB).

2.6. UV irradiation

For UV irradiation, HEK293T or HeLa cells were seeded at 70% confluence and then subjected to different doses of UV- C irradiation for different times, using a UV source ENF-260C/FE Spectroline® UV lamp (Spectronics Corporation, Westbury, NY, USA). The UV dose used for all experiments was 50 J/m^2, followed by a recovery period of for 2 h, in a CO_2 incubator at 37°C and protected from further light.

2.7. Flow cytometry and EdU labeling for fluorescence microscopy

Flow cytometry was used for cell cycle and apoptosis analysis. For cell cycle analysis, cells were fixed with 70% ethanol, stained with 100 µg propidium iodide and analyzed by flow cytometry using FACS Canto II (Becton Dickinson). For apoptosis determination we used the FITC Anexin V Apoptosis Detection Kit (BD Pharmingen™) and followed the manufacturer's instructions. To detect DNA synthesis (S -phase), cells were incubated for 2 hours with 30 µM thymidine analog EdU (5-ethynyl-2'-deoxyuridine). EdU incorporation was detected using click Chemistry reaction and azide labelled with Alexa Fluor 488, according to manufacturer instructions (Click-iT Edu Image kit, Invitrogen). The images were captured by confocal laser scanning microscope (Leica TCS SP8) and analyzed and processed using Image J 1.43

2.8. Immunofluorescence microscopy

To immunofluorescence assay, cells irradiated or not with UV-C were fixed and permeabilized with 3.7% formaldehyde solution (Sigma-Aldrich, F1635), containing 0.2% Triton X-100 in PBS 1X, then blocked for 30 min in blocking buffer containing 3% bovine serum albumin, and 0.1% Triton X-100 (Sigma-Aldrich) in PBS. The cells were incubated for 1 hour with primary antibodies, diluted in blocking buffer, containing mouse anti-phospho-Histone H2A.X (EMD Millipore 05-636; 1:500 dilution). Then, cells were washed with 1x PBS and incubated with secondary antibody chicken anti-mouse Alexa Fluor 488 - (dilution 1:500) for 40 min. Hoechst was used to stain DNA.- Data image were collected on a Zeiss LSM 780 NLO Confocal Microscope (Carl Zeiss AG, Germany) using 40X or 100X lens. Series of Z stack images were captured from 0.5 µm thick sections and images were processed using Image J software program (http://rsb.info.nih.gov/ij/).

3. Results and discussion

3.1. Nek7 is associated to CAK complex and phosphorylates Mat1

In response to genotoxic stress, cells protect their genomes integrity by activating a conserved DDR pathway that coordinates DNA repair and cell cycle progression [26, 27]. Clear roles for Nek7 in regulating the cell cycle [4, 28], as well as for Mat1 in the cell cycle response to DNA-damaging agents have been established [29, 30, 21, 14]. However, there has so far been no evidence to support an involvement of Nek7 in DNA damage-induced cell-cycle regulation, via Mat1. We have earlier reported a yeast two-hybrid screens for Nek7 that resulted in the identification of the CAK assembly factor Mat1 as a Nek7 interactor [13]. This interaction prompted us to investigate if Nek7 can associate with the others CAK components and if Mat1 is phosphorylated by Nek7, to obtain clues about possible new functions for this protein. Toward this end, we performed pull down assay using recombinant Nek7 as bait and endogenous CDK7, Cyclin H and Mat1 as prey from HEK293T cells. As shown in the Western blots in Fig. 1A, Nek7 was able to associate specifically with all CAK components, but not with the control protein RAR, indicating that it can be found in association with the CAK

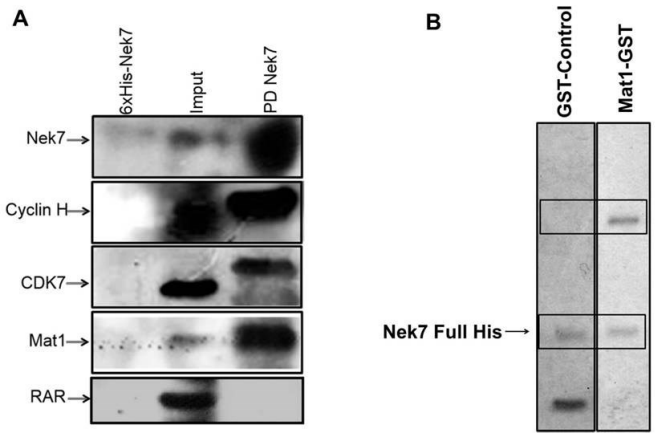

Figure 1 | Nek7 is associated to CAK complex and phosphorylates Mat1. A) Imuno blot detection (anti Nek7, Cyclin H, CDK7, Mat1 or RAR) of co-precipitated proteins from pull-down (PD) of recombinant Nek7 (6xHis-Full-Length-Nek7) from Hek293T (detection of endogenous Cyclin H, Cdk7 and Mat1). RAR was used as a negative control. The pull-down assay results are based on three independent experiments. B) Autoradiography of P_{32} labelled proteins. Recombinant 6xHis-Nek7 phosphorylates GST-full-length-Mat1, but not the negative control protein GST.

complex. In order to determine whether the CAK assembly factor Mat1 can serve as a substrate for Nek7, an in vitro kinase assay was performed, using recombinant Nek7 and Mat1. The results show that Mat1 is indeed phosphorylated by Nek7 (Fig. 1B) in vitro. These findings are in line with our previous report of Mat1 being an Nek7 interactor and substrate [13] and supply further evidence that different CAK components could be regulated by Nek7 to mediate CAK functions in the context of the cell cycle, apoptosis and potentially DNA repair.

Thus, we speculated that the interaction and phosphorylation of Mat1 by Nek7 may influence the equilibrium between the free ternary CAK and the core TFIIH-related CAK. In this context, Nek7 could contribute to regulating Mat1 functions, including mechanisms that involve the substrate specificity of CDK7 [31, 32], cyclin H expression regulation [18] or Mat1 participating in nucleotide excision repair [30,14].

3.2. Nek7 and Mat1 regulate UV-induced DDR during S-phase

Recent data have shown that CAK proteins are involved in DNA repair mechanisms, particularly in UV damage response, with Mat1 being rapidly recruited to the injury site [14]. UV radiation is among the most frequent causes of DNA damage to cells that lead to a DNA damage repair, cell cycle arrest and apoptosis [22]. Since Mat1 has been previously implicated in DNA damage repair [14], our detection of the Nek7-Mat1 interaction and phosphorylation, raised the hypothesis that both proteins may cooperate in the UV-induced DDR.

To explore this connection, Mat1 or Nek7 were depleted by shRNA from HeLa cells (Fig. 2A-B), irradiated or not with 50 J/m2 UV and examined by immunofluorescence staining of phosphorylated histone γH2AX (Suppl. Fig.1), a key player of the UV-induced DDR that when detected in the form of nuclear foci, is considered to be a marker of formation of DNA double-strand breaks (DSBs) [23, 24]. The results show that the UV treatment promoted a nuclear accumulation of γH2AX suggesting that Nek7 or Mat1 inhibition somehow is involved in either inducing or increasing the UV-derived DNA damage. Increased levels of γH2AX may result in the activation of DDR and the phosphorylation of the ATM and ATR kinases, which in turn activate the mitotic checkpoints Chk1, Chk2 and the pro-apoptotic p53 protein, to induce cell cycle arrest with accumulation of cells in the S-phase and apoptosis [35]. Thus the possible relation of Nek7/Mat1 with other proteins involved in this pathway should be better characterized in the future.

Under these conditions, Nek7 or Mat1 depleted cells (Fig.2 A,B) were submitted to cell cycle analysis using flow cytometry. According to Fig. 2D, in the non-irradiated condition, after Nek7 depletion the percentage of cells in the S-phase (replicating cells) presented an increase of about 5%, while upon Mat1 depletion, there was an increase of 30% when compared to shRNA-control transfected cells. When these cells were UV irradiated (Fig. 2E) we observed an increase of the percentage of cells in the S-phase of 18.5% (Nek7 depletion) or 28.8% (Mat1 depletion). Therefore, Nek7 or Mat1 inhibition led to decreased accumulation of cells in the S-phase. These findings support the notion, that interference with Nek7 or Mat1 may regulate DNA replication during S-phase upon DNA damage and can potentially affect cell proliferation.

To further explore this hypothesis, we decided to investigate whether Nek7 and Mat1 have roles in cell proliferation. To this end, cells over-expressing Nek7 wild-type or Nek7 or Mat1-depleted (Fig. 2A and 2B, respectively) were irradiated or not with 50 J/m2 of UV-C, and then labeled with EdU (5-ethynyl-2'-deoxyuridine). Then, cell proliferation was assessed by fluorescence microscopy and flow cytometry.

Fig. 3 (A and B) shows that after UV light exposure all cell types tested showed a significant decrease in proliferation. However, in the cells with Nek7-overexpression, the proliferation was significantly higher and in the cells with Nek7 (Fig.3B) or Mat1 (Fig. 3D) depletion, the EdU incorporation was significantly reduced, or abolished, after UV light exposure (Fig. 3A-D). These results are consistent with Nek7 or Mat1 regulating the S-phase (Fig 2D and E), and suggest that inhibition of Nek7 and mainly Mat1 affect cellular proliferation mostly after in DNA damage conditions.

3.3. Nek7 and Mat1 trigger UV-induced apoptosis

Based on the above results, we decided to check if Nek7 or Mat1 could, eventually, increase UV-induced apoptosis as a consequence of both the cell cycle arrest and increase in the number of cells in the S-phase. Therefore, we examined whether UV-induced apoptosis is affected by Nek7 and Mat1. To this end, Nek7 or Mat1-depleted cells as well as wild-type Nek7 or "kinase dead" Nek7 (Nek7K63A and Nek7K63/64A) overexpressing cells (Fig. 2A-C), were irradiated with 50 J/m2 of UV-C to be submitted to an apoptosis assay, using flow cytometry.

Indeed, Nek7 or Mat1 depletion induced a significant increase of the cell´s percentage undergoing apoptosis (Fig. 4A and 4B, respectively and Suppl. Fig. 2A and B), with a higher tendency to additionally increase after UV irradiation.

Interestingly, there was a decreased percentage of cells undergoing apoptosis upon UV irradiation after wild-type Nek7 over-expression, in opposition to what is observed after "kinase dead" Nek7 over-expression (Fig. 4C and Suppl. Fig.3). These results are in line with the UV-induced S -phase arrest following wild-type Nek7-overexpression (Fig. 3 A-B) and suggest that Nek7 and its kinase activity are important to protect the cells of UV-induced apoptosis.

Together, these findings may indicate that Nek7 and Mat1

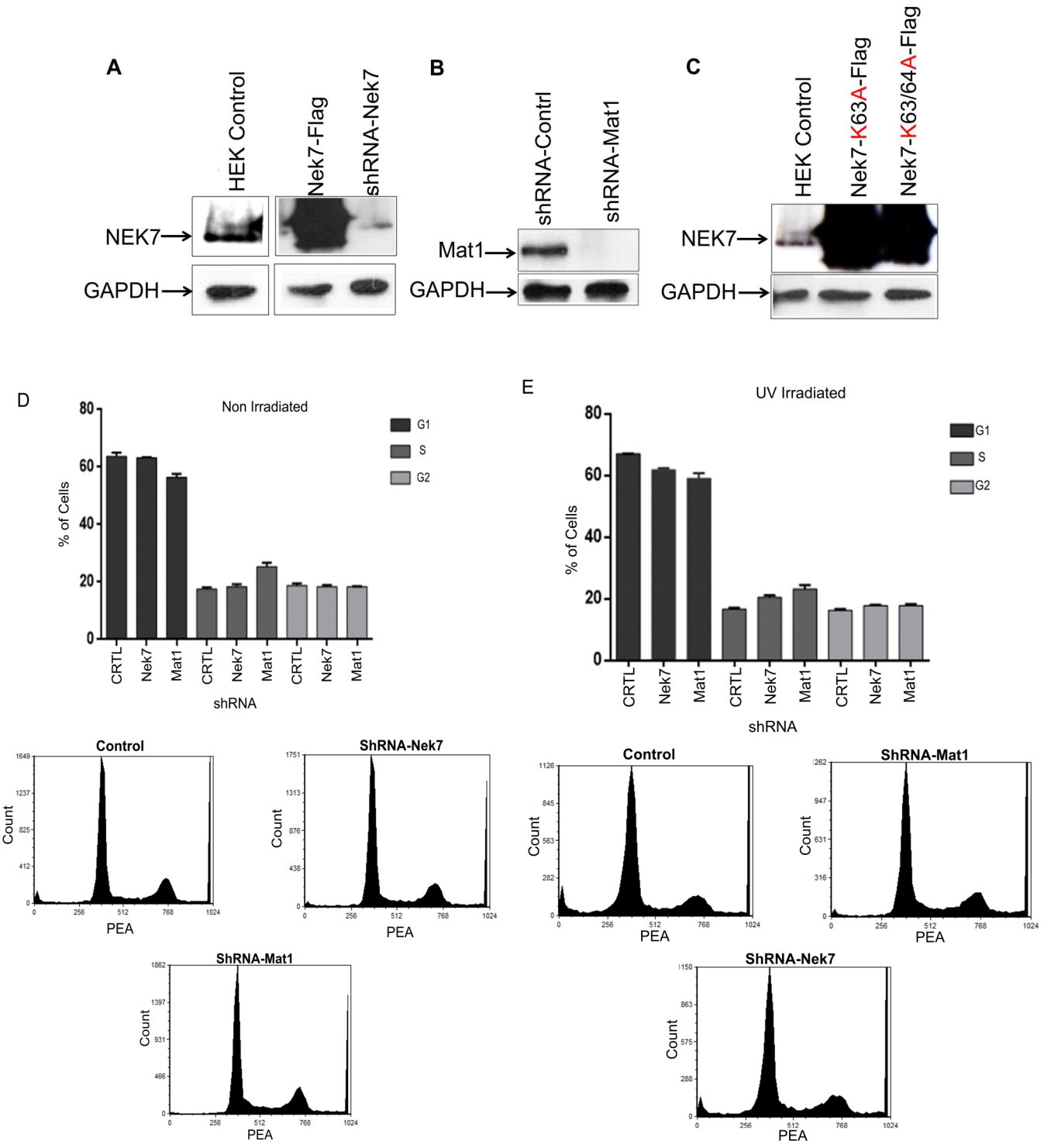

Figure 2 | Nek7 and Mat1 regulate S-phase upon induction of DDR by UV irradiation. A) Western blot (anti-Nek7 imuno blot) of Hek293T cells showing the Nek7 wild type, over-expression of Nek7-Flag and also Nek7 depletion by shRNA; B) Western blot of Hek293T cells showing Mat1 depletion by shRNA; C) Western blot of Hek293T cells showing the over expression of Nek7 with two different point mutations in the kinase domain (Nek7-K63A and Nek7K63/64A). GAPDH antibody was used as loading control. D-E) Graphs represent the FACS analysis showing the cell cycle distribution - The bars correspond to the different phases of the cell cycle (G1, S, G2 fases) for control cells depleted for Nek7 or Mat1 in normal conditions and after UV irradiation respectively. D. Statistical significant differences between columns 1 and 3 and between columns 4 and 6: $P < 0.05$ calculated using two-way ANOVA, Bonferroni post-test (GraphPad Prism). $P< 0{\cdot}05$ calculated using two-way ANOVA, Bonferroni post-test (GraphPad Prism). Histograms represent the FACS analysis showing the cell cycle distribution. Studies were performed in cells control, Nek7 and Mat1 depleted cells (shRNA-control, shRNA-Nek7, shRNA-Mat1) irradiated or not with 50 J/m2 of UV-C light and recovered for 2 hours.

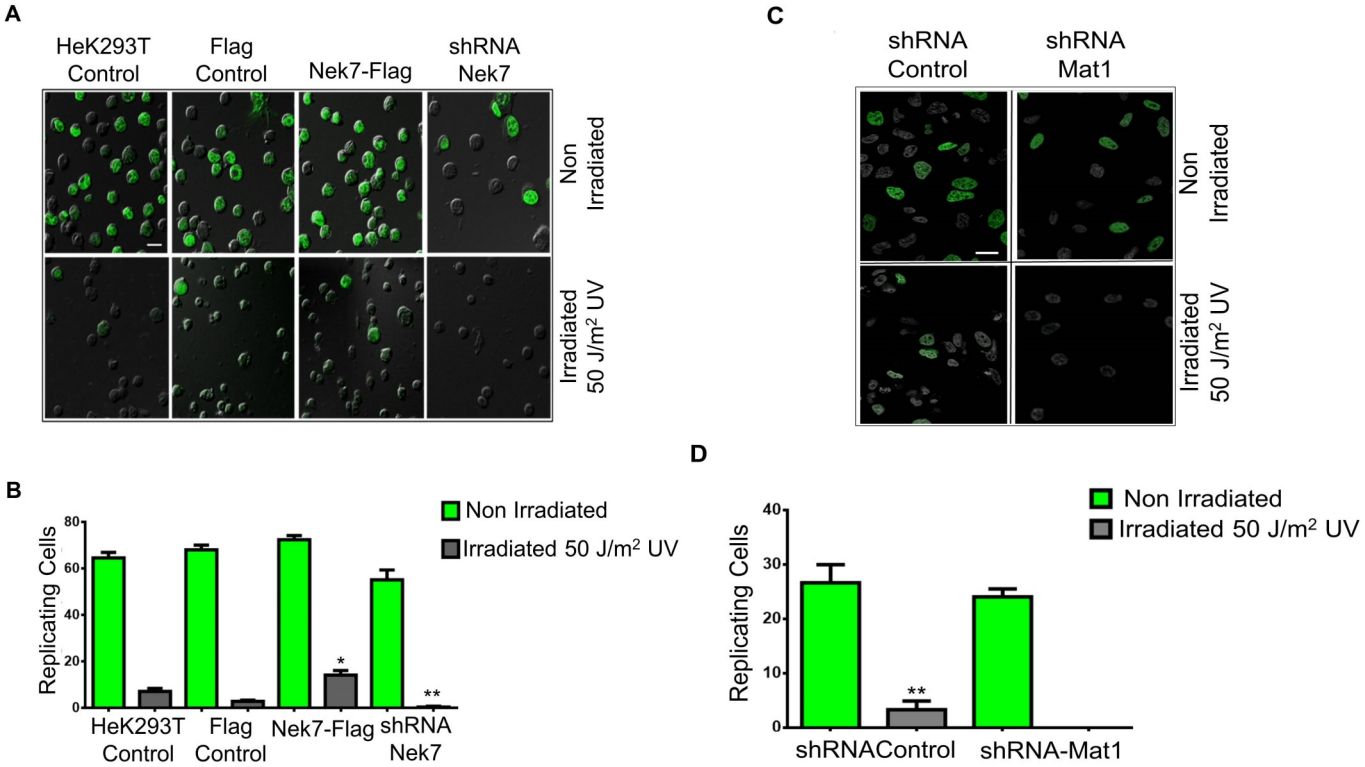

Figure 3 | Nek7 and Mat1 modulate cell proliferation: A) Non transfected Hek293T (control), over-expressing Nek7 (Nek7-Flag) and depleted for Nek7 (shRNA-Nek7), were irradiated or not with 50 J/m2 of UV-C light and recovered for 2 hours. After this the cells were stained with Alexa Fluor 488-EdU for cell replication analysis by fluorescence microscopy. Cell stained green are replicating. B) Representative graphs of the experiment shown in A, indicating the percentage of replicating cells. Cultures irradiated with UV (grey bars) or not (green bars). (*, **) indicate P < 0,05; calculated using two-way ANOVA, Bonferroni post-test (GraphPad Prism). C) The same experiment show in B was done for Mat1 depleted cells (shRNA-Mat1). D) Representative graphs of the experiment shown in C, indicating the percentage of replicating cells. Cultures irradiated with UV (grey bars) or not (green bars). (**) Indicates P < 0,05 calculated using two-way ANOVA, Bonferroni post-test (GraphPad Prism).

cooperate in the DDR and regulate a cell-cycle arrest and even apoptosis to prevent of accumulation of further mutations, genome instability and hence carcinogenesis. This adds with Nek7/Mat1 another pair of protein players to the eukaryotic cells arsenal to respond to DNA damage by activating a network of biochemical pathways that enable damage recognition and initiate responses leading to repair, apoptosis or senescence [25].

In line with these results, the wild-type Nek7 over-expression reduced the apoptosis, in opposition to what is

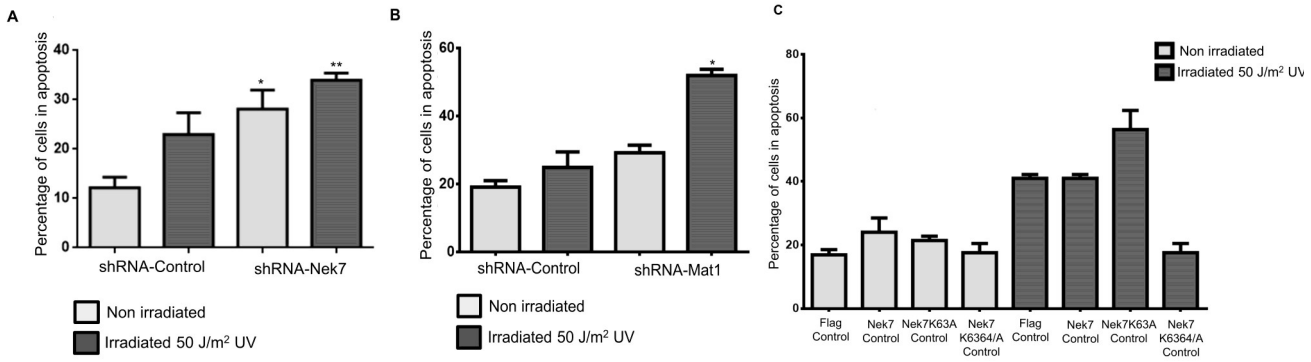

Figure 4 | Nek7 and Mat1 mediate UV-induced apoptosis. shRNA control cells, Nek7 or Mat1 depleted cells and cells over-expressing wide-type Nek7 or "kinase dead" (Nek7-K63A and Nek7K63/64A) were irradiated or not with 50 J/m2 of UV-C light and recovered for 2 hours. After this, the Hela cells were stained with propidium iodide and annexin-FITC and analyzed by flow cytometry. Graph corresponds to percentage of apoptosis cells. A) Graphs represent the percentage of apoptosis cells obtained by FACS analysis of control cells (shRNA-Control) or cells depleted by Nek7 (shRNA-Nek7); B) Control cells (shRNA-Control) and cells depleted of Mat1 (shRNA-Mat1); and C) Control cells and wild type Nek7 or kinase dead over-expressed (Nek7-K63A and Nek7-K63/64A). (*, **) Indicate P < 0,05 calculated using two-way ANOVA, Bonferroni post-test (GraphPad Prism).

observed for inactivated (without kinase activity) Nek7 over-expression. Taken together these findings support the hypothesis that Nek7 and Mat1 can affect cell cycle progression by promoting selective accumulation of cells in the S phase, upon DNA damage.

This study provides evidences that cell cycle arrest and apoptosis mediated through Nek7 and Mat1 may dependent on the activation of the DDR pathway, suggesting that Nek7 and Mat1 carry out their functions by temporarily halting cell proliferation, perhaps by engaging in specific cell cycle checkpoints or may alternatively affect CDK7s activity toward p53 [33, 19, 34]. The latter possibility suggests that triggering of p53 may then indirectly result in activating the response to DNA damage followed by DNA repair.

4.Conclusion

Here, we report that Nek7 interacts with the CAK complex and may cooperate with Mat1 in the S-phase regulation, in response to DNA damage. Thus, we speculated that the interaction and phosphorylation of Mat1 by Nek7 may influence the equilibrium between the free ternary CAK and the core TFIIH-related CAK. In this context, Nek7 could contribute to regulating Mat1 functions, including mechanisms that involve the substrate specificity of CDK7 [31, 32], cyclin H expression regulation [18] or Mat1 participating in nucleotide excision repair [30,14].

Our results show that Nek7 or Mat1 depletion led to a decreased cell proliferation, accumulation of cells in the S-phase and ultimately to an increase in apoptosis.

Alternatively, Nek7 and Mat1 may cooperate in mechanisms to eliminate cells by switching from non-successfully repaired UV–induced DNA damage to apoptosis. This model is consistent with the previous reports that over-expression of a kinase-defective form of Nek7 or its silencing result in both higher mitotic index and higher apoptosis [4,28]. Mat1 defective cells fail to enter S phase [36], and RNA antisense depletion of Mat1 in rat aortic smooth muscle cells induced a G1 arrest followed by apoptosis [37]. Both Nek7 and Mat1 gene knock-out in mice severely affect mitosis and lead both to early embryonic lethality [7, 18]. The details of the functional mechanism involved in the Nek7-Mat1 interplay, yet need to be determined in future experiments.

Briefly, our findings support the notion that Nek7 may cooperate with Mat1 in the signaling pathways that govern the cell cycle regulatory machinery, including DDR, S-phase progression and apoptosis, and thereby can constitute an important natural barrier against cellular transformation and tumorigenesis.

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Refrences

[1] G. V. Meirelles; A. M. Perez.; E. E. Souza.; F. L. Basei; P. F. Papa; T. D. Melo Hanchuk et al. World J. Biol. Chem. 5 (2014) 141–160.
[2] J. Roig, A. Mikhailov, C. Belham, J. Avruch, Nercc1. Genes Dev. 16 (2002) 1640-58.
[3] C. Belham, J. Roig, J. A. Caldwell, Y. Aoyama, B. E. Kemp, M. Comb, J. Avruch. J Biol Chem. 278 (2003) 34897-909.
[4] N. Yissachar, H. Salem, T. Tennenbaum, B. Motro. FEBS Lett. 580 (2006) 6489-95.
[5] S. Kim, K. Lee, K. Rhee. Biochem Biophys Res Commun. 360 (2007) 56-62.
[6] S. Cohen, A. Aizer, Y. Shav-Tal, A. Yanai, B. Motro. Biochim Biophys Acta. 1833 (2013) 1104-13.
[7] H. Salem, I. Rachmin, N. Yissachar, S. Cohen, A. Amiel, R. Haffner, L. Lavi, B. Motro. Oncogene 29 (2010) 4046−4057.
[8] R. Wang, Y. Song, X. Xu, Q. Wu, C. Liu. Transl Oncol. 15 (2013) 626-32.
[9] J. Xu, L. Lu, L. Li. Acta Biochim Biophys Sin. 48 (2016) 966-968.
[10] H. Shi, Y. Wang, X. Li, X. Zhan, M. Tang, M. Fina, L. Su et al. Nat Immunol. 17 (2016) 250-8.
[11] L. Zhou, Z. Wang, X. Xu, Y. Wan, K. Qu, H. Fan, Q. Chen, X. Sun, C. Liu. Oncotarget. 7 (2016) 18620-30.
[12] R. Tan, S. Nakajima, Q. Wang, H. Sun, J. Xue, J. Wu, et al. Mol Cell. 65 (2017) 818-831.
[13] E. E. de Souza, G. V. Meirelles, B. B. Godoy, A. M. Perez, J. H. Smetana, S. J. Doxsey, et al. Proteome Res 13 (2014) 4074-90.
[14] Q. Zhu, G. Wani, N. Sharma, A. Wani. DNA Repair. 11 (2012) 942-50.
[15] M. Matsuno, H. Kose, M. Okabe, Y. Hiromi. Genes Cells 12 (2007) 1289–1300.
[16] D. Hoogstraten, A. L. Nigg, H. Heath, L. H. Mullenders, R. Van Driel, J. H. Hoeijmak, et al. Mol. Cell 10 (2002) 1163–1174.
[17] H. Patel, R. Abduljabbar, C. F. Lai, M. Periyasamy, A. Harrod, C. Gemma, et al. Clin Cancer Res. 14 (2016).
[18] D. J. Rossi, A. Londesborough, N. Korsisaari, A. Pihlak, E. Lehtonen, M. Henkemeyer, et al. EMBO J. 20 (2001) 2844-56.
[19] L. J. Ko, S. Y. Shieh, X. Chen, L. Jayaraman, K. Tamai, Y. Taya, C. Prives, Z. Q. PanQ. Mol Cell Biol 17 (1997) 7220-9.
[20] L. Wu, P. Chen, C. H. Shum, C. Chen, L. W. Barsky, K. I. Weinberg, A. Jong, T. J. Triche. Mol Cell Biol. 21 (2001) 260-70.
[21] J. O Fuss, J. A. Tainer. DNA Repair. 10 (2011) 697-713.
[22] F. R. de Gruijl, H. J. van Kranen, L. H. Mullenders. J. Photochem. Photobiol. 63 (2001) 19–27.
[23] H. Zhao, F. Traganos, Z. Darzynkiewicz. Cytometry A. 77 (2010) 285-93.
[24] W. T. Chen, A. Alpert, C. Leiter, F. Gong, S. P. Jackson, K. M.

Miller. Mol Cell Biol. 33 (2013) 111–126.
[25] E. Mladenov, S. Magin, A. Soni, G. Iliakis. Semin Cancer Biol. 37 (2016) 51-64..
[26] Jackson, P. Stephen, B. Jiri. Nature 461 (2010) 1071–78.
[27] Akaike, Y. Kuwano, K. Nishida, K. Kurokawa, K. Kajita, S. Kano, K. Masuda and K. Rokutan. Oncogene (2014), 1–11.
[28] L. O'Regan, A. M. Fry. Mol Cell Biol. 29 (2009) 3975-90.
[29] J. P. Adamczewski, M. Rossignol, J. P. Tassan, E. Nigg, V. Moncollin, J. M. Egly. The EMBO journal. 15 (1996) 1877–84.
[30] A. Zhovmer, V. Oksenych, F. Coin. Scientific World Journal. 10 (2010) 633-43.
[31] M. Rossignol, I. Kolb-Cheynel, J. M. Egly. EMBO J. 16 (1997) 1628-37.
[32] K. Y. Yankulov, D. L. Bentley. EMBO J. 16 (1997) 1638-46.
[33] H. Lu, R. P. Fisher, P. Bailey, A. J. Levine. Mol Cell Biol 17 (1997) 5923-34.
[34] E. Schneider, M. Montenarh, P. Wagner. Oncogene 17 (1998) 2733-41.
[35] A. Celeghin, S. Giunco, R. Freguja, M. Zangrossi, S. Nalio, R. Dolcetti, A. De Rossi. Cell Death and Disease 7 (2016).
[36] S. Zhang, Q. He, H. Peng, N. Tedeschi-Blok, T. J. Triche, L. Wu. Cancer Res. 64 (2004) 2977-83.
[37] L. Wu, P. Chen, J. J. Hwang, L. W. Barsky, K. I. Weinberg, A. Jong, V. A. Starnes. J Biol Chem. 274 (1999) 5564-72.