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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

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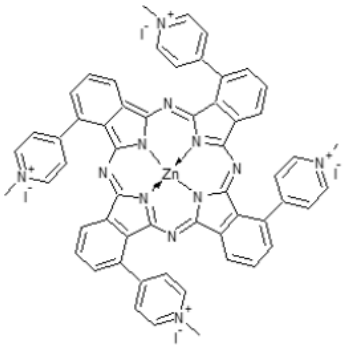
ABSTRACT

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

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

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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.

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Sequencing Applied to Modern Clinical Microbiology

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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.

Acknowledgments:

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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

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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.

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Opinions and regularity conclusions on drug combination to prevent resistance

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

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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.

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Gold nanoparticles as alternative therapy for antibiotic-resistant bacterial strains

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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.

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

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

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

Upshots of a decennium of antimicrobial resistance proteomics

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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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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

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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.

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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

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

Combating Antibiotic Resistance: Glyconanomaterials, Nanoantibiotics and Drug Repurposing

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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.

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

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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

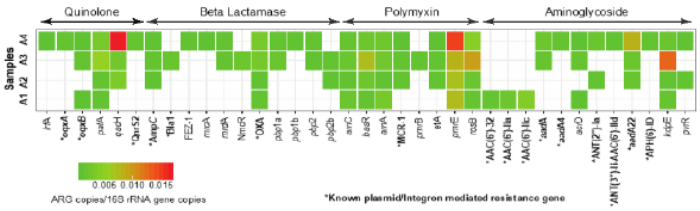

Figure 1: *Enterobacteriaceae* Antibiotic Resistance Profile

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

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

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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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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

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

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

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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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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

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

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

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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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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

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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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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

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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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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

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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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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]

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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].

Acknowledgments:

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

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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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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

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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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Identification of multiresistant methicillin-resistant *Staphylococcus aureus* (MRSA) in wild hares (*Lepus granatensis*) from 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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Linezolid resistant *cfr*-positive methicillin-resistant *Staphylococcus aureus* isolated from infected diabetic foot ulcers

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

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

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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.

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Contending with the Black Swans of Resistance

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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.

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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

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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.

Acknowledgments:

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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

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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.

Acknowledgments:

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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

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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.

Acknowledgments:

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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

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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.

Acknowledgments:

Authors are grateful to O. Golovko, B. Vetoschnikova and M. Pleskacheva for the technical assistance. Authors are grateful to O. Ivanova and S. Karabanov for providing MDR Enterococcus spp. isolates.

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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

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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

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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*

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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/).

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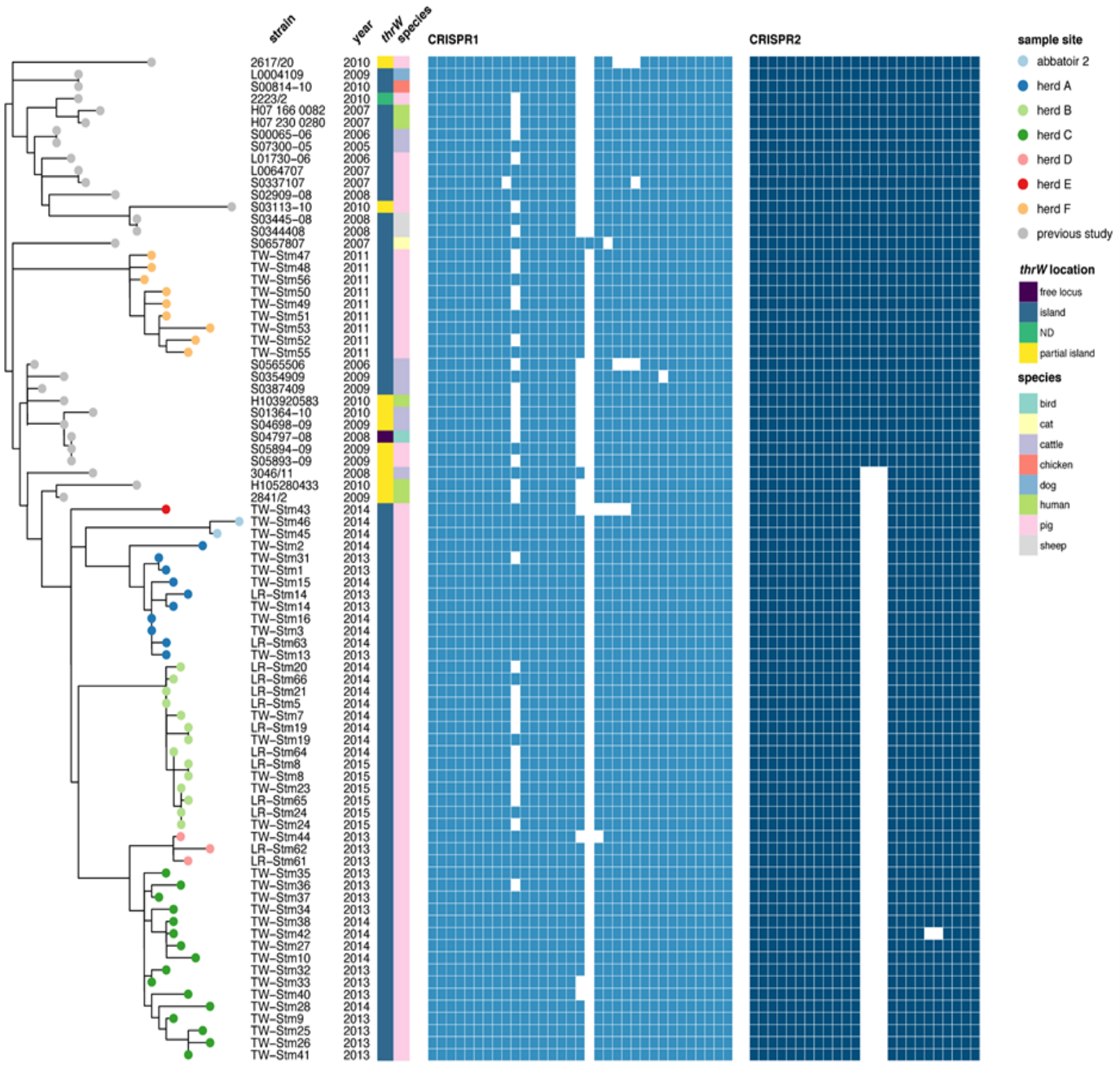

Figure 1: Phylogenetic tree and CRISPR array comparison of Australian porcine S. 1,4,[5],12:i:- isolates (colored tips) and European S. 1,4,[5],12:i:- isolates (grey tips) from various sources.

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

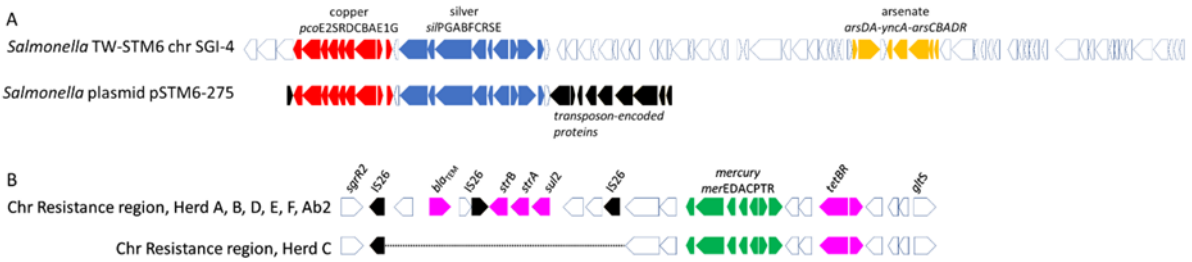

Figure 2: Comparison of the arrangement of heavy metal resistance genes of SGI-4 and pSTM6-275 (A). Comparison of the chromosomal resistance regions of isolates from different herds (B).

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.

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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

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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

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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.

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