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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE IV INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE (IC²AR 2021)

Dual activity of PNGM-1 pinpoints the evolutionary origin of subclass B3 metallo-β-lactamases

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ABSTRACT

Metallo-β-lactamases (MBLs) are able to hydrolyze almost all β-lactams including carbapenems and thereby to confer critical antibiotic resistance threats [1]. However, the evolutionary origin of MBLs remains unknown. To find antimicrobial resistance gene(s) predating the use of antibiotics through metagenomics, functional screening of a metagenomic library from the deep-seep sediments of Edison Seamount in the pre-antibiotic era was performed [2]. Molecular, biochemical, and cell-based experiments (MIC values) were performed to define a PNGM-1 (Papua New Guinea Metallo-β-lactamase) as a novel subclass B3 MBL. Further analyses of PNGM-1 were done as follows: (i) functional, (ii) evolutionary phylogenetic (MEGA X), (iii) X-ray crystal structure analyses of PNGM-1 and PNGM-1 mutant (H91A, H93A, D95A, H96A, or H257A), and (iv) their comparison with structural properties of other MBLs (proteins containing the αββα-fold with β-lactamase activity: AIM-1, GOB-18, and FEZ-1) and structurally representative MBL fold proteins (proteins having αββα-fold without β-lactamase activity: three tRNase Zs) of the MBL superfamily. In addition to biochemical and cell-based results, the hydrolyzed form of β-lactam ring of doripenem shown in the active site structure of doripenem-bound H257A showed that PNGM-1 is a unique subclass B3 MBL. Our phylogenetic analysis suggests that PNGM-1 yields insights into the evolutionary origin of subclass B3 MBLs. We reveal the structural similarities between tRNase Zs and PNGM-1, and demonstrate that PNGM-1 has both MBL and tRNase Z activities, suggesting that PNGM-1 is thought to have evolved from a tRNase Z. We also show kinetic and structural comparisons between PNGM-1 and other proteins including subclass B3 MBLs and tRNase Zs. These comparisons revealed that the B3 MBL activity of PNGM-1 is a promiscuous activity and subclass B3 MBLs are thought to have evolved through PNGM-1 activity. Based on our results, we suggest that subclass B3 MBLs arose through an evolutionary trajectory (tRNase Z → PNGM-1→ subclass B3 MBLs) and the origin of subclass B3 MBLs is a tRNase Z [3]. These evolutionary processes help us to understand where subclass B3 MBL genes came from and predict the future evolution of MBL genes.

Keywords: Antimicrobial resistance, Subclass B3 metallo-β-lactamase, tRNase Z, Dual activity, Structure and evolutionary origin

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Dietary synbiotic supplementation as alternative to antibiotic growth promoters on broiler performance and health status

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ABSTRACT

Due to antibiotic resistance concerns, probiotics and synbiotics have gained considerable interest in poultry feeding as alternatives to antibiotics. The objective of this experimental study was to evaluate the potential of a multispecies poultry specific synbiotic product in comparison and/or in combination to some antibiotics used in poultry feeds on broiler chicken's performance and health status. A total of 1260 Ross 308 male one-day-old broiler chicks were randomly assigned to 7 dietary treatments with 6 replicates for each dietary treatment and 30 chicks per replicate. The trial was conducted for a period of 42 days in 4 feeding phases (Starter days 0-10, Grower days 11-24, Finisher I days 25-35 and Finisher II days 36-42). The dietary treatments included a corn-soybean based control diet without any additives (T1) and the treatment diets containing either Bacitracin, (100 ppm, T2), Colistin (10 ppm, T3), Synbiotic (PoultryStar® me, 0.5 kg/t, T4) or a combination of Synbiotic and Bacitracin (T5), Synbiotic and Colistin (T6) and Synbiotic, Bacitracin and Colistin (T7). The study revealed that synbiotic application (T4) in broiler feed resulted in significantly higher body weight gain (BWG) than its combination with Bacitracin during the critical period of rearing from hatch to day 10 ($P<0.05$). None of the other treatments showed any remarkable improvement in BWG, feed intake (FI) or feed conversion ratio (FCR), compared to the single synbiotic application (T4) during the overall rearing period from 0-42 days ($P<0.05$). FCR tended to be improved in the synbiotic supplemented group (T4, 1.87) compared to the control group (T1, 1.93) during the overall trial duration. Likewise, bird mortality was also lower in the synbiotic group (T4, 1.11%) in comparison to the control group (T1, 2.78%). In conclusion, supplementation of PoultryStar® me (synbiotic application) in broiler chicken feeding could serve as a replacement and an effective alternative to the use of antibiotic growth promoters like Bacitracin and Colistin, especially during the early critical rearing period. The synbiotic can serve this purpose alone without any combination with AGP's like Bacitracin and/or Colistin and thus could be cost effective for broiler production.

Treatment groups[1]	BWG (g/bird)	FI (g/bird)	FCR	Mortality (%)
T1	192.7 [ab]	263.5	1.36	0.00
T2	193.1 [ab]	272.6	1.41	0.00
T3	199.1[a]	271.4	1.36	0.00
T4	198.5[a]	270.2	1.36	0.00
T5	186.1[b]	272.4	1.46	0.00
T6	199.7[a]	267.6	1.34	0.00
T7	193.2 [ab]	265.6	1.37	0.00
p -value	0.0337	0.6047	0.1492	0.00
SEM	1.27	1.51	0.013	0.00

Table 1 | Dependences of iron migration energy change on US intensity. Temperature of US loading is 340 K.
[a,b] Means with dissimilar letters in a column varied significantly ($p < 0.05$) 1 T1= No additives in feed, T2 = Bacitracin, T3 = Colistin, T4 = Synbiotic (PoultryStar® me), T5 = Synbiotic + Bacitracin, T6 = Synbiotic + Colistin, T7 = Synbiotic + Bacitracin + Colistin. BWG = Body Weight Gain, FI = Feed Intake, FCR = Feed Conversion Ratio, SEM = Standard Error of Means

Keywords: Forensic Anthropology, Forensic Genetics, Paleopathology, Spondyloarthropathies, HLA-B27, SNPs

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Centrifugation of boar spermatozoa through low density Porcicoll to separate them from bacteria does not affect fertility after insemination

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ABSTRACT

Boar ejaculates become contaminated during semen collection. Therefore, antibiotics are added to semen extenders for artificial insemination, as required by governmental directives [1], to maintain sperm quality during storage and minimise the risk of causing an infection in the inseminated sow. However, this non-therapeutic use of antibiotics could be contributing to antimicrobial resistance, since both the sow and the environment are exposed to these antibiotics. Colloid centrifugation separates spermatozoa from most of the bacteria in boar semen [2] and can be done in 500 mL tubes to process whole boar ejaculates [3]. The objective of the present study was to test the fertility of sperm samples processed by Single Layer Centrifugation through low density Porcicoll in a pilot artificial insemination trial. Boar ejaculates were extended in Beltsville Thawing Solution (BTS). Each ejaculate was split, with half being prepared by Single Layer Centrifugation in 500 mL tubes and the other serving as control [3]. After centrifugation, the sperm pellets were resuspended in BTS; antibiotics were then added as the inseminations were being done on a commercial farm. In total, 17 sows were inseminated in the control, group and 12 in the SLC group. The numbers of sows farrowing and litter data are shown in Table 1.

Table 1 | Outcome of inseminations with boar semen prepared by Single Layer Centrifugation through 20% Porcicoll or uncentrifuged controls

Sperm Sample (n)	Farrowing	Total born	Live born	Born dead	Mummified
CONTROL (17)	13	14.53±5.1	13.6±5.1	0.92±1.0	1.08±1.8 *
SLC 20P (12)	9	15.96±3.4	15.3±2.9	0.77±0.88	0.66±0.71 *

Note: * p=0.022

There were no differences in pregnancy rates or prolificacy when factoring in the boar effect, with the exception of the number of mummified piglets, which was lower in the SLC 20P group than in controls. Although the sample size is small, these results show that boar spermatozoa processed by SLC with 20P are capable of generating pregnancies after artificial insemination. Therefore, a larger artificial insemination trial is warranted, since this method of processing could enable boar semen samples to be processed and stored without the addition of antibiotics if the regulations were changed.

Keywords: Antimicrobial resistance, Single Layer Centrifugation, Low density Porcicoll, Pregnancy rate, Litter size

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Challenges of the genotypic detection of antimicrobial resistance for stewardship and infection control

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ABSTRACT

Antimicrobial resistance continues to be an issue of global concern. Rapid molecular assays to identify antimicrobial resistance genes in pure colonies or to identify bacterial species and resistance genes in parallel directly from patient specimens can be used to optimize antibiotic treatment regimens. The results of the tests are also important for guiding infection control programs to prevent spread of multidrug-resistant organisms. The key questions regarding molecular tests are the positive and negative predictive values of the results. If the molecular test is positive for a resistance gene, the likelihood that the organism is phenotypically resistant is high but if the molecular test is negative for a resistance gene, how confident can one be that the isolate is phenotypically susceptible to the target drug? For methicillin-resistant Staphylococcus aureus both positive and negative predictive values are very high. For gram-negative organisms, predicting susceptibility or resistance to third-generation cephalosporins and carbapenems based on detection of a limited set of beta-lactamase genes can be improved by limiting reporting to a key group of "bacterial species-resistance gene-antibiotic" combinations. This is particularly important for interpreting the results of large syndromic panels that identify multiple bacterial species but have a limited set of antimicrobial resistance genes. The utility of whole genome sequencing for predicting susceptibility and resistance to beta-lactam drugs can also be improved by adding detection of mutations for porin gene regulation and chromosomal AmpC-type beta-lactamases to the interpretation algorithm. Thus, molecular methods can significantly enhance antimicrobial stewardship efforts, but this requires the thoughtful development of reporting algorithms up front.

Keywords: Molecular diagnostics, MRSA, Carbapenems, Syndromic panels, WGS

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Examining the synergy of antimicrobial peptides and peptide mimics with traditional antibiotics

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ABSTRACT

There is an increasing need to develop new therapeutic options to treat bacterial diseases as bacteria are becoming increasingly resistant to traditional antibiotics. We have been developing antimicrobial peptides and peptide mimics as alternative antibiotics. Our antimicrobial peptides melimine and Mel4 have been assessed in human clinical trials when bound to contact lenses. However, we have also shown that these peptides can be destroyed by proteases. Therefore, we have developed and studied several different types of peptide mimics. These peptide mimics are based upon N-substituted glycine or alkyl-guanidine and are resistant to proteases. In the current study, we examined the ability of these antimicrobial peptide mimics to synergise with traditional antibiotics. The minimum inhibitory concentration (MIC) of the peptide mimics and traditional antibiotics was measured according to CLSI guidelines. The fractional inhibitory index (FICI) was measured as the ratio of the MICs of the antimicrobials alone and combined. FICIs of ≤0.5 indicated synergy, 0.6-1.0 an additive effect, 1.1-4.0 indifference and >4.0 antagonism. Bacteria (*Pseudomonas aeruginosa* (antibiotic resistant and MDR strains), *Staphylococcus aureus* (MRSA and MDR)) and *Candida auris* were screened. The median MICs (mM) for peptides, N-substituted glycine or alkyl-guanidine mimics were: *P. aeruginosa* 86.5, 13.0, 20.6; *S. aureus* 17.4, 2.0, 5.1; C. auris 17.4, 3.6, 15.6. The FICI of bacterial strains when tested in combination with ciprofloxacin or gentamicin ranged from 0.3 to 2.0. No combination produced an antagonistic response, and certain combinations with certain strains produced synergism. These data indicate that the peptide mimics based upon N-substituted glycine or alkyl-guanidine can be potentially used to treat antibiotic resistant infections in combination with traditional antibiotics. Furthermore, these N-substituted glycine or alkyl-guanidine peptide mimics may also be useful as agents to treat infections caused by the often multidrug-resistant yeast *C. auris*.

Keywords: Antibiotic resistance; Candida auris; Peptide mimic; Synergy

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New Phage Pastilles Against Antibiotic Resistant Bacterial Infection of Oral Cavity

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ABSTRACT

Every year millions of people suffer from infections of oral cavity and upper respiratory tract infections (tracheitis, pharyngitis, angina, ...) which are caused mostly by *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp. and Pathogenic *E. Coli*[1]. Today the pharmaceutical market is supplied with antimicrobial preparations such as, pastilles being active only at the initial stage of the infection and antibiotics really having a broad range of activity, but due to a lot of side-effects, their administration is not desirable in children, pregnant women, for immunocompromised patients. In addition, emergence of the bacteria being resistant to all available antibiotics is a big problem for medicine today[2]. We demonstrate a new form of Phage preparation-Phage pastilles, that has high antibacterial and anti-inflammatory activity, able to solve above-mention problem[3]. Phage Pastille is a new preparation that contains wide host range phages as an active antimicrobial substance and also anti-inflammatory and antiviral active substances as Salvae Oleum and Extractum Salvae. A total 8 phages with high host range were selected (among them 2 phages are *Staphylococcus* spp. specific, 2-*Streptococcus* spp. phages, 2 -*Enterococcus* spp. phages and 2 -*E. coli* phages). These phages have ability to remain active at 55 0C for at least 30min. Sequencing and genome annotation results confirmed that those phages have lytic life style and do not contain any toxic/drug resistant genes and can be used for therapeutic remedy. Phage remain active with coexistence of the pastille substances (Salvae Oleum, extractum salvae, izomalt, menthol). For stability and long-term shelf life of phage pastilles, 0.7% gelatin and 1% sucrose were added in composition. Small -scale batches of Phage pastilles were produced, and antibacterial activity of created phage pastilles was confirmed on 430 bacterial strains shown lysis in 91%. Due to its safety the Phage Pastille will be recommended to be used for both prophylactic and therapeutic purposes and can be used by any person regardless of age or physical condition.

Keywords: Phage Therapy, Antibiotic resistance, Pastilles, Phage preparation, Phage Pastilles

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The *PmrA* / *PmrB* mutations do not contribute to colistin resistance in *mcr-1-negative E. coli* isolates isolated from Russian farm animals

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ABSTRACT

The *mcr-1* gene is known to determine colistin resistance in gram-negative bacteria. The *mcr-1* gene encodes phosphatidylethanolamine transferase, which modifies lipid A of the bacterial cell wall. Mutations in some chromosomal genes can also provide colistin resistance in *E. coli* [1]. These genes include the *PmrA* / *PmrB* genes, which are also involved in lipid A modification [2]. This work aimed to study mutations in the *PmrA* / *PmrB* genes in *mcr-1* negative colistin-resistant isolates of *E. coli*. We collected samples from chickens, turkeys, pigs, and cows raised in farms in 5 different Russian regions. Using the serial broth microdilution method, we evaluated colistin resistance of *E. coli* isolated from these samples. In 92 isolates, we determined the presence of the *mcr-1* gene by PCR; we applied the Illumina Miseq targeted sequencing to the *PmrA* / *PmrB* genes. We assessed *PmrA* / *PmrB* mutations functionally by the Provean online tool [3]. Twenty-eight isolates of *E. coli* (30.4%) were resistant to colistin, while only 9 of them (9.8%) possessed the *mcr-1* gene. Whole-genome sequencing of five *mcr-1-negative* colistin-resistant isolates did not reveal any other *mcr* genes. In *mcr-1-negative* colistin-resistant isolates, we found amino acid substitutions S129G, A64S, G144S in the *PmrA* gene and H2R, D283G, V351I, N358Y in the *PmrB* gene. However, the susceptible isolates contained the same amino acid substitutions with the same frequency ($p>0.05$, X2-test). The T171M, A242T, A360V substitutions in the *PmrB* were found only in resistant isolates, but they referred to the highly polymorphic region of the *PmrB* protein. The only substitution N264K located in the conservative region of the *PmrB* protein was found in an mcr-1-positive resistant isolate. Thus we did not observe any mutations in the chromosomal *PmrA* / *PmrB* genes reliably associated with colistin resistance in *E. coli* isolated from farm animals in Russia.

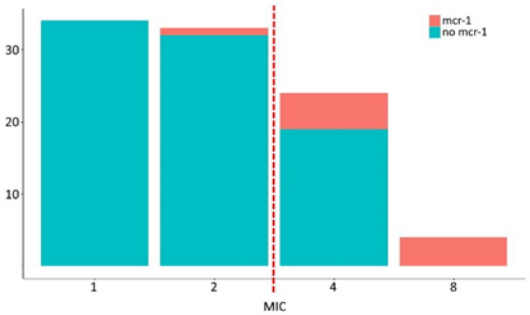

Figure 1 | Only one-third of colistin-resistant E. coli isolates possessed the mcr-1 gene.

Keywords: Colistin-resistant E. coli; PmrA / PmrB genes; mcr-1 negative isolates, targeted sequencing; farm animals

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Development of a fast antibiotic sensitivity test using flow cytometry

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ABSTRACT

Even today, antibiotic resistance is still a major and continuously growing problem worldwide. Due to the increasing occurrence of antibiotic resistance, bacterial infections are more difficult to treat. Actions such as, prevention, surveillance and new antimicrobial agents are vital in order to tackle bacterial resistance. To control antibiotic resistance, new and rapid laboratory methods are necessary. Antimicrobial susceptibility testing (AST) methods became essential and are still routinely used. However, despite their importance, the conventional AST methods (disk diffusion and photometry) are time consuming and can take up to 48 hours. Development of an effective and reliable tool that can determine antibiotic sensitivity faster, will ensure a better treatment for patients and a reduced incidence of antibiotic resistance.

A study was performed using flow cytometry to quantify growth of Escherichia coli ATCC 25922 and amoxicillin‐resistant E. coli ATCC 35218 in the presence and absence of antibiotics. Therefore, the E. coli strains were incubated at 37 °C in TSB medium in the presence and absence of amoxicillin (16 µg/ml) and amoxicillin/clavulanic acid (16 µg/ml). A sample of the incubated strains was taken every 15 minutes for 3 hours and analyzed with the flow cytometer and spectrophotometer. Comparable experiments were performed with different bacteria, such as Klebsiella pneumoniae ATCC 700603, Staphylococcus aureus ATCC 29213 and methicillin-resistant Staphylococcus aureus (MRSA) in the presence and absence of several antibiotics (amoxicillin, oxacillin and erythromycin). Furthermore, a method was developed to determine the minimum inhibitory concentrations (MIC's) for these antibiotics using flow cytometry. For the E. coli strains the MIC's were determined, using different concentrations of amoxicillin and amoxicillin/clavulanic acid (2-fold serial dilutions starting from 32 µg/ml). During incubation at 37 °C samples were taken every 30 minutes for 6 hours and analyzed with the flow cytometer.

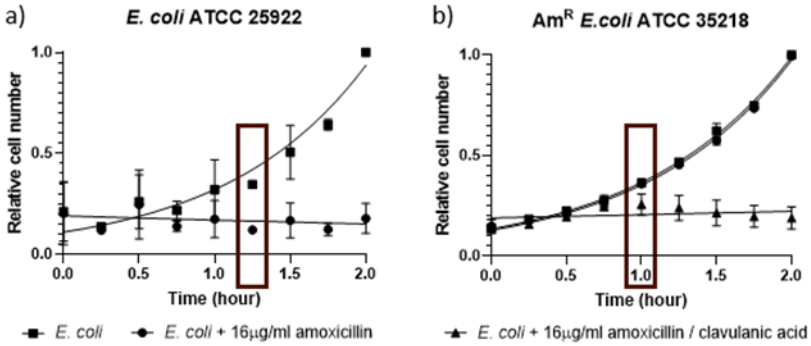

Figure 1 | Antimicrobial susceptibility growth test: a) E. coli ATCC 25922 in the presence and absence of amoxicillin, b) amoxicillin‐resistant E. coli ATCC 35218 in the presence and absence of amoxicillin and amoxicillin/clavulanic acid (mean ± SEM; n ≥ 3).

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The antimicrobial susceptibility growth test showed exponential growth of E. coli ATCC 25922 after 1.25 h ($p \leq 0.05$, $n \geq 3$), while there was no growth in the presence of amoxicillin (see figure 1a). For the amoxicillin‐resistant E. coli strain (ATCC 35218), susceptibility for amoxicillin with clavulanic acid could be observed after 1 h, while there was still growth in the presence and absence of amoxicillin (figure 1b).

The determination of the MIC's, using flow cytometry, showed that after 4 hours the lowest concentration of amoxicillin that inhibits the growth of E. coli ATCC 25922 was 8 µg/ml. For amoxicillin‐resistant E. coli ATCC 35218 the MIC was 16 µg/ml amoxicillin/clavulanic acid.

In this proof-of-principle study using E. coli, a fast antibiotic susceptibility testing method was developed. Using the flow cytometer, resistance can be detected at least 2 hours sooner compared to the conventional antibiotic resistance tests used in clinical laboratories. Similar results were obtained for the other bacteria and antibiotics. The further development and use of this fast antibiotic sensitivity test could lead to an increasing use of the appropriate antibiotic and thereby reducing the incidence of antibiotic resistance.

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Trends in outpatient antibiotic consumption for urinary infections in Croatia: self-evidence and controversies

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ABSTRACT

Due to the rapid development of resistance, health systems around the world are facing challenges in terms of rising treatment costs, hospitalizations, and mortality. Care for patients with resistant infections is becoming increasingly difficult also in the primary care segment. In the EU countries, outpatient antimicrobial spending is monitored in the the ESAC Net project, and resistance in the EARSS project. Annual outpatient antibiotic consumption in 2018 in Croatia was 17 DDD/TID. The analysis of trends in antibiotic consumption for urinary infections included nitrofurantoin, fosfomycin, co-trimoxazole and fluoroquinolones. Outpatient consumption in the period 2005-2018 decreased by about 5.4% (from 3.35 to 3.17 DDD/TID, mostly at the expense of co-trimoxazole (decreased by 70%), while the prescribing of quinolones increased by 41%. The use of ciprofloxacin increased by 200% - from 0.25 to 0.75 DDD/TID, despite the fact that its prescribing was restricted (only on the recommendation of a hospital specialists) in order to reduce consumption and maintain efficiency. Nitrofurantoin use increased by 88% - from 0.43 to 0.81 DDD /TID. In 2018 the three fluoroquinolones (norfloxacin, ciprofloxacin and levofloxacin) with 1,71 DDD/TID were used almost twice as much as nitrofurantoin (0.81 DDD/TID). The use of co-trimoxazole decreased by 70% (from 1.65 in 2005 to 0.5 DDD/TID in 2018), while prescribing of fosfomycin (registered in Croatia in 2012) was almost negligible (0.07 DDD/TID). Ciprofloxacin utilization increased almost 2,2 time faster compared with nitrofurantoin (200% against 88%), which probably have contributed to the increase in its resistance on a country level (unsusceptibility of E. coli rose from 7% in 2000 up to 21% in 2018, or by 200% and of P. mirabilis from 3% in 2000 up to 31% in 2018, or by 900%).

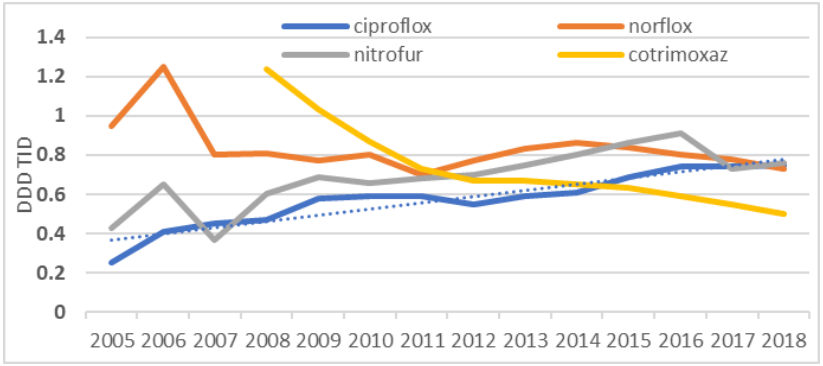

Figure 1 | Outpatient utilization of urinary antibiotics in Croatia (2005 – 2018).

Keywords: Antibiotic consumption, Antibiotic resistance, Primary care, Urinary infections

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE IV INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE (IC²AR 2021)

Do We Still Need New Antibiotics to Treat Multidrug-Resistant Enterobacteriaceae?

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ABSTRACT

Although a lack of new antibiotics plagued the infectious disease community in the early 2000s, we have recently seen a resurgence of novel agents to treat multidrug-resistant (MDR) Enterobacterales. These agents have been developed in response to urgent pleas by groups such as the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO).

Thirteen new antibacterial agents have been licensed since 2014, with eight of these exhibiting activity against MDR Gram-negative bacteria. Notable among these new agents are beta-lactamase inhibitor (BLI) combinations with novel inhibitor structures, the diazabicyclooctanones avibactam and relebactam and the boronic acid derivative vaborbactam. Combinations with these inhibitors effectively inhibit serine beta-lactamases, including the KPC serine carbapenemases responsible for major outbreaks of infections due to MDR Enterobacterales. In addition, cefiderocol, a novel siderophore cephalosporin that utilizes an iron uptake mechanism for entry into Gram-negative pathogens, has expanded coverage to include many MDR Enterobacterales that produce metallo-carbapenemases such as NDM-1 as well as serine carbapenemases. Non-beta-lactam-containing agents that have been recently approved include the aminoglycoside plazomicin and the tetracyclines eravacycline and omadacycline, all of which were developed to avoid many of their class-specific resistance mechanisms.

As a result of the introduction of these agents, many previously "untreatable" carbapenem-resistant infections are now able to be cured by beta-lactam-containing drugs, or by new drugs in other chemical classes that have targeted these infections. Investigational agents in late clinical development to expand the treatment of MDR Gram-negative infections include additional BLI combinations and several polymyxins. However, as experience has shown us in the past, introduction of new agents will only lead to new resistance mechanisms. Thus, the search for new agents must continue.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE IV INTERNATIONAL CAPARICA CONFERENCE IN ANTIBIOTIC RESISTANCE (IC²AR 2021)

Ionic liquids on the rescuing of conventional antimycobacterial drugs

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Extended-Abstract

The incidence of infections by nontuberculous mycobacteria (NTM) is increasing worldwide, mainly those caused by Mycobacterium avium complex (MAC) species [1]. NTM are opportunistic pathogens that infect immunocompromised patients, namely those infected with HIV, with cancer or who were subject to a transplant. NTM are highly infectious and cause persistent infections due to their ability to easily form aerosols, to settle as biofilms and to resist to harsh environments, like chlorinated water [2]. In the host, mycobacteria proliferate inside phagocytic cells, such as macrophages. There, they multiply inside small vacuoles and control the intracellular vesicular trafficking inhibiting the phagosome-lysosome fusion, which allows them to escape the lysosomal acidic environment and to have access to nutrients [3]. NTM infections manifest primarily as pulmonary diseases, but can also affect other regions of the body, like the central nervous system, and cause lymphadenitis, which is the most common NTM-associated disease in immunocompetent children [4]. The treatment basis of slow-growing NTM, in which MAC is included, is a macrolide. Clarithromycin or azithromycin are the usual options. A regimen of monotherapy with macrolides is, however, very dangerous as it will often lead to drug resistance and consequent treatment failure. Thus, a three-drug macrolide-based regimen with ethambutol and a rifamycin, which usually lasts from 6 to 12 months, is the recommended treatment. The addition of a fourth drug to the regimen, like aminoglycosides or a fluoroquinolone, can be important in more severe cases and is essential in cases of macrolide-resistant MAC [5]. A very long multi-drug regimen like this, results in several issues to the patients, which decreases the probability of treatment success. It is thus urgent to find a new strategy to treat mycobacterial infections, including the repurposing of old drugs [6]. Ionic liquids (ILs) are organic salts made by the combination of two molecules with opposite polarities. Their remarkable physical and chemical properties contributed for their extensive use as green-solvents, improving the performance and safety of chemical procedures, as well as vehicles in sensors and drug delivery systems [7]. Recently, ILs have gained much attention in the area of drug development as antimicrobial agents, since they have shown improved solubility and bioavailability when compared to clinically approved drugs [8]. The right combination of cations and anions can provide innovative compounds that help combat resistance issues. The aim of our work is to evaluate the capacity of ILs based on conventional antimycobacterial drugs to inhibit the viability and growth of M. avium in axenic culture and inside bone marrow-derived macrophages (BMM). We are assessing if the activity and toxicity of these compounds are improved by being in the IL form instead of being administered individually or in combination. Our results show that ILs derived from each of two fluoroquinolones, ofloxacin or norfloxacin, and the antimycobacterial drug clofazimine cause a more significant decrease in the extracellular and intracellular mycobacterial viability than the fluoroquinolones administered individually. Moreover, the ILs are less toxic to the host cells than clofazimine. Another pair of ILs, which combine one classical antimalarial drug, chloroquine or primaquine, with the anti-tuberculosis drug aminosalicylic acid, also shows promising results: the ILs are more active against M. avium growing inside BMM than the three parental drugs

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by themselves. Therefore, our data encourage us to continue combining conventional anti-NTM antibiotics with molecules active against other pathogens in an IL form as a way to enhance their activity, improve pharmacological issues and combat resistances. In the future, we aim to test these ILs in more complex in vitro models of infection, such as biofilms and in vitro granulomas, taking advantage of fluorescent and bioluminescent reporter strains of M. avium, in order to better predict their clinical outcome and reduce the use of animals in preliminary drug testing.

Keywords: *Mycobacterium avium*, *In vitro* infection, Repurposing old drugs, Ionic liquids

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