**Rapid Fire Presentations II**

**Targeting Protein-Protein Interactions for Novel Antibiotic Discovery**

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**Background:** Antimicrobial resistance emerges to become a serious threat to antimicrobial therapy. Novel antimicrobial agents are urgently required to eradicate current drug-resistant bacteria and are expected to ideally suppress new resistance generation. Protein-protein interactions (PPI) commonly exist in bacteria. Drugs targeting PPI would theoretically increase the difficulty of mutations and fitness costs, therefore reducing the resistance generation.

**Methods:** We studied the PPI between RNA polymerase (RNAP) and the housekeeping sigma factor, which is essential for RNAP holoenzyme formation and the initiation of RNA synthesis. Through structure-based drug design, we developed a series of benzoic acid derivatives to mimic the structure of sigma factor at the critical binding site with RNAP. This series of small molecule compounds was coined sigmacidin to acknowledge the drug design strategy mimicking sigma factor and the benzoic acid structure.

**Results:** Sigmacidin compounds showed inhibitory activity to interrupt the RNAP-sigma PPI and reduce RNA synthesis and toxin release. They also exhibited excellent antimicrobial activity against Gram-positive bacteria including antibiotic-resistant strains such as MRSA and VRSA. The 30-day serial passage did not show resistance generation. In vivo pharmacokinetic studies demonstrated oral availability, and the drug efficacy was also proven in an MRSA-infected peritoneal sepsis model.

**Conclusions:** Targeting PPI is a valid strategy for antimicrobial drug discovery. Sigmacidin is currently under preclinical studies for further drug development.

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**The Urinary Microbiome as a Source of Novel Antimicrobials against Uropathogenic Escherichia coli**

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**BACKGROUND:** Urinary tract infections (UTIs) are one of the most common bacterial infections worldwide. As global incidences of UTIs caused by multidrug resistant bacteria continue to increase, so too does the demand for novel antimicrobial therapies. Due to its relatively understudied nature, the urinary microbiome represents a niche with an untapped source of potentially novel antimicrobials (e.g., bacteriocins). Improvements to bacterial culturing and sequencing techniques have highlighted these potential alternative treatments and control strategies to target antibiotic resistant uropathogenic *E. coli* (UPEC). **METHODS:** Expanded quantitative urine culture
(EQUC) was used to culture bacterial isolates from mid-stream urine samples. Urinary isolates were then tested for their ability to inhibit a bank of five clinically relevant UPEC strains using deferred antagonism assays. Biochemical characterisation, together with genomic analysis, was used to identify and characterise the isolates to species level, and to identify the putative antimicrobial agents. **RESULTS:** A large bank of 260 bacterial isolates from mid-stream urine samples were screened against five clinically relevant UPEC strains resulting in 24 shortlisted isolates displaying antimicrobial activity. Further analysis and characterisation for bacteriocin production resulted in 4 shortlisted isolates. Preliminary bioinformatic screening in BAGEL4 for bacteriocin gene clusters (BGCs) resulted in the discovery of 15 putative bacteriocin operons. After manual annotation five were considered for further investigation. These included putative genes for microcin, colicin and colicin E1 bacteriocin production. Further investigation indicated that these putative bacteriocin associated areas of interest (AOIs) revealed two microcin precursor peptides (McmA and MchB), three S-type pyocin domain-containing proteins and two colicin E1 proteins with the potential to inhibit UPEC growth. **CONCLUSION:** Given that UTIs caused by UPEC are becoming more difficult to treat due to increasing antibiotic resistance, the putative antimicrobials described herein represent a viable potential alternative to antibiotics for the control and prevention of UTIs.

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Session Title: Rapid Fire Presentations II  
Publishing Title: HoloMoA: Digital Inline Holographic Microscopy to Rapidly and Cost-effectively Determine the Mechanism of Action (MoA) of Antimicrobials  
Author Block: Z. Sedaghat, B. Courbon, D. K. Mercer, C. Guyard, C. Vedrine, S. Dixneuf; Bioaster, Lyon, France  
Background: The global crisis of antimicrobial resistance requires solutions from different scientific approaches. Determination of the MoA of antimicrobials, especially novel antimicrobial drug candidates, is highly desirable from a regulatory standpoint, yet can be a time-consuming and expensive process. A rapid, robust, inexpensive and versatile method for determination of the MoA of antimicrobials is highly desirable. **Methods:** We have developed a novel, label-free technology based on digital inline holographic microscopy (DIHM), coupled to deep-learning analysis called HoloMoA. Time-lapse DIHM images are captured and analysed longitudinally to determine phenotypic changes. *Escherichia coli* ATCC 25922, with or without antibiotics, was monitored using time-lapse DIHM at 37°C for 2 h using, in most cases, the MIC of the antibiotic. We included 22 antibiotics in our database, corresponding to 5 MoA classes and control samples without antibiotic. We used 3 replicates per antibiotic to ensure reproducibility. Holograms were acquired every 3 min and reconstructed by a back-propagation algorithm based on Rayleigh Sommerfeld diffraction theory, while twin image artefacts were removed through a modified Latychevskaia algorithm. We developed deep learning models to classify these time-series among 6 classes (5 antibiotic MoA classes + control). Our chosen model was based on Convolutional Neural Networks. The time dimension of the data was analysed using 3-dimensional
convolutional kernels (CNN3D). **Results:** HoloMoA was able to determine the MoA of known antimicrobials active against *Escherichia coli* with >89% accuracy. Morphological characteristics, including filamentation, bulging, growth retardation and swelling were classified using a 3D Convolutional Neural Network. **Conclusions:** HoloMoA was able to determine the MoA of known antimicrobials against *E. coli* with >89% accuracy. The functionality of HoloMoA is currently being extended to Gram positive bacteria and will later be applied to other pathogens. Accuracy will be improved taking into account antibiotics with more than one MoA and off-target effects. Importantly, the tools developed will also be able to determine if novel antimicrobials have a new MoA.

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**Session Title:** Rapid Fire Presentations II

**Publishing Title:** Optimization of Darobactins Improving the Antibiotic Activity by Structure and Activity Guided Biosynthetic Engineering

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**Abstract Body:**

Background The antibiotic crisis with emerging mortality rates caused by multidrug resistant pathogens urgently asks for the development of novel antibiotic classes breaking existing resistance mechanisms in clinically relevant strains. **Methods** We aimed to optimize darobactin, a new antibiotic class attacking an unique target site at the outer-membrane protein BamA. We have initially heterologously produced and genetically engineered improved darobactin derivatives by modifying the terminal amino acids to achieve enhanced antibacterial activity against Gram-negative strains such as *P. aeruginosa* (0.125 µg mL⁻¹). **Results and Conclusion** Based on a Cryo-EM structure-guided approach we achieved a further optimization of antibacterial activity of novel analogues up to 128-fold compared to native darobactin A against carbapenem-resistant *A. baumannii* strains (0.0625 - 0.5 µg mL⁻¹) without any sign for cytotoxicity.

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