

Control Number: 2023-A-44-ASM-ESC

Session Title: **Poster Presentations Session II**

Publishing Title: **Susceptibility Profiles of Baseline Gram-negative Pathogens from CERTAIN-1, a Phase 3 Study comparing Cefepime-taniborbactam to Meropenem in Adults with Complicated Urinary Tract Infection (cUTI)**

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**Background:** Taniborbactam is an investigational  $\beta$ -lactamase inhibitor that restores cefepime (FEP) activity against FEP-, carbapenem-, and multidrug-resistant (MDR) Enterobacterales and *Pseudomonas aeruginosa* producing serine- and metallo- $\beta$ -lactamases. Cefepime-taniborbactam (FTB) was superior to meropenem (MEM) for the primary composite (microbiologic and clinical) endpoint at test of cure in adults with cUTI in the CERTAIN-1 study (NCT03840148). We compared susceptibility to FEP, FTB, and MEM among Enterobacterales and *P. aeruginosa* recovered at baseline. **Methods:** MICs were determined by broth microdilution (CLSI M07) for baseline pathogens from patients in the extended microbiologic intent-to-treat population (Enterobacterales and/or *P. aeruginosa* at  $\geq 10^5$  CFU/mL in urine against which  $\geq 1$  study drug had activity [FTB MIC  $\leq 16$   $\mu$ g/mL; MEM MIC  $\leq 2$   $\mu$ g/mL (Enterobacterales) or  $\leq 4$   $\mu$ g/mL (*P. aeruginosa*)]). Phenotypic subsets included ESBL, and FEP-, multidrug-, and carbapenem resistance (CLSI M100). **Results:** Taniborbactam decreased the FEP MIC<sub>90</sub> by  $\geq 1,024$ -fold (to 1  $\mu$ g/mL) against FEP-resistant, ESBL, and MDR subsets of Enterobacterales and by  $\geq 128$ -fold (to 8  $\mu$ g/mL) against carbapenem-resistant Enterobacterales (Table). FTB at  $\leq 16$   $\mu$ g/mL inhibited 66.7%, 71.4% and 100% of FEP-, multidrug-, and carbapenem-resistant *P. aeruginosa*, respectively. Higher percentages of Enterobacterales and *P. aeruginosa* isolates, regardless of resistance phenotype, were inhibited by FTB compared to MEM.

**Table.** Cefepime (FEP), Cefepime-taniborbactam (FTB), and Meropenem (MEM) Susceptibility Summary for Baseline Isolates of Enterobacterales and *P. aeruginosa* from Patients in the Extended Microbiological Intent-to-Treat (extended MicroITT) Population

Pathogen/phenotype (n)	MIC <sub>90</sub> or MIC range ( $\mu$ g/mL)			%Susceptible		
	FEP	FTB	MEM	FEP	FTB*	MEM
Enterobacterales overall (437)	512	0.25	0.12	73.5	99.8	96.8
Cefepime-resistant (106)	>512	1	2	0	99.1	88.7
ESBL (126)	>512	1	1	8.7	99.2	90.5
MDR (167)	>512	1	0.5	34.7	99.4	91.6
Carbapenem-resistant (10)	>512	8	64	10.0	100	0
<i>P. aeruginosa</i> overall (23)	32	16	16	69.6	91.3	82.6
Cefepime-resistant (6)	32-512	4-32	0.25->64	0	66.7	50.0
MDR (7)	16-512	4-32	0.25->64	0	71.4	57.1
Carbapenem-resistant (5)	4-512	4-16	0.5->64	40.0	100	20.0

Abbreviations: ESBL, extended spectrum  $\beta$ -lactamase (aztreonam, ceftazidime and/or cefepime MIC  $\geq 2$   $\mu$ g/mL); FEP, cefepime; FTB, cefepime-taniborbactam; MDR, multidrug-resistant (resistant to  $\geq 1$  agent in  $\geq 3$  classes); MEM, meropenem.\*In the absence of breakpoints, %Susceptible for cefepime-taniborbactam reflects % of isolates inhibited at  $\leq 16$   $\mu$ g/mL.

**Conclusions:** Taniborbactam potentiated FEP activity against resistant isolates of Enterobacterales and *P. aeruginosa* from patients in the CERTAIN-1 cUTI study. These results are consistent with the ability of taniborbactam to restore

# FEP activity against most isolates of FEP-, multidrug-, and carbapenem-resistant gram-negative pathogens producing serine- and metallo-β-lactamases in nonclinical studies.

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**Control Number:** 2023-A-46-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **Exploring SAR-endolysins To Fight Multidrug-resistant Gram-negative Bacteria**

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**Abstract Body:**

**Background:** Treating infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) is a challenge. Bacteriophage-derived lytic enzymes have been studied as a potential solution for treating these infections. Despite its potential, studies have shown that the outer membrane of GNB hampers the exogenous action of endolysins. However, recent research has demonstrated that endolysins with signal-anchor-release (SAR) domains can permeate the outer membrane of GNB. Therefore, this study aimed to determine the antimicrobial activity of SAR-endolysins against GNB strains. **Methods:** Based on genomic and metagenomic data, we previously described 66 SAR-endolysins. Combined with previously published data, there are 119 non-redundant SAR-endolysins described in the literature. We clustered these putative antimicrobial proteins using principal component analysis (PCA) based on biochemical properties and domain presence/absence. Then, we assessed the antimicrobial potential of one of the representative clusters against five bacterial species, including three MDR strains. The representative SAR-endolysin was cloned into pET29b, transformed into *Escherichia coli* Lemo 21(DE3), and purified using the Fast His-Tagged Protein Purification (Zymo Research). The *in vitro* antimicrobial activity was determined by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests. **Results:** SAR domains were found in glycoside hydrolase families 19 (GH19), 24 (GH24), 25 (GH25), and 108 (GH108). These endolysins were clustered into eight PCA groups. To assess the strength of the genomic/metagenomic screening and the potential antimicrobial activity of SAR-endolysins against Gram-negative pathogens, we chose one representative SAR-endolysin from the largest PCA group (103 out of 109; 94.50%) and evaluated the MIC and MBC for the GNB strains. SAR-endolysin LysKpV475 showed bacteriostatic activity ranging from 8.125 µg.ml<sup>-1</sup> for *P. aeruginosa* ATCC 27853 and 32.50 µg.ml<sup>-1</sup> for *K. pneumoniae* ATCC BAA-2146 (MDR) and *E. cloacae* P2224 (MDR). Bactericidal activity was observed for *P. aeruginosa* ATCC 27853 (32.50 µg.ml<sup>-1</sup>) and *P. aeruginosa* P2307 (65.00 µg.ml<sup>-1</sup>). **Conclusions:** These results highlight the importance of genomics and metagenomics in discovering new antimicrobials. Further studies are needed to determine the role of the SAR domain and the polyhistidine tag in the antimicrobial activity of SAR-endolysins. This study highlights the potential of SAR-endolysins to fight MDR-GNB.

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**Control Number:** 2023-A-56-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **CZ-02s are a New Class of Antibiotics, with a New Mechanism-of-Action to Treat Multidrug-Resistant Bacteria**

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**Abstract Body:**

**Introduction**The CZ-02 class of antibiotics targeting the bacterial ribosomal P-site are being developed for treatment of multi-drug resistant (MDR) bacterial infections, including those caused by agents of bio warfare/terrorism. These unique antibacterials act on a clinically un-drugged binding site of the most validated intracellular antibacterial target (the ribosome) with a new mechanism of action (P-site inhibitors) and does not show cross-resistance to other antibiotics, including protein synthesis inhibitors. **Methods** Antibacterial activity was assessed using the broth microdilution method to determine MICs, assay kits were used to evaluate inhibition of protein synthesis and cytotoxicity *in vitro*. Resistance frequency was determined by adding AIIPS to molten cation-adjusted Mueller Hinton agar at 2, 3, and 5X the MIC and incubated for 48h at 35°C. Pharmacokinetics were evaluated in infected and uninfected mice (male and female) administered a single subcutaneous dose with blood and epithelial lining fluid samples collected (4 mice/CZ-02) at 8 time points. Efficacy in a plague pneumonia model was accomplished by administering compounds at 4 doses q6h to mice that were exposed to *Y. pestis* CO92. For toxicology studies, rats were administered CZ-02s twice/day for 7 days. Clinical signs were monitored and histopathology performed for tissues from high-dose animals. **Results** CZ-02s exhibit potent antibacterial activity against a wide variety of pathogens, including biothreats, significant ELF penetration with outstanding potency *in vivo* against *Y. pestis* CO92 and tolerability in animals administered multiple doses over 7 days. **Conclusion** CZ-02s are being advanced as medical countermeasures against Tier 1 select agents, showing high-level potency

against *Y. pestis*, *F. tularensis* and *B. mallei* with sub- $\mu\text{g/mL}$  MICs. ADME profiling demonstrates good metabolic stability, low volume of distribution, excellent bioavailability with very low protein binding. PK shows favorable tissue distribution along with efficacy in mouse pneumonia models with low propensity for resistance and safety after multiple days of repeat dosing.

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**Control Number:** 2023-A-67-ASM-ESC  
**Session Title:** **Poster Presentations Session II**  
**Publishing Title:** **Extracellular Vesicles Exhibit Antibiofilm Effects on *Pseudomonas aeruginosa***  
**Author Block:** T. Solomon, S. Jay; Univ. of Maryland Coll. Park, College Park, MD

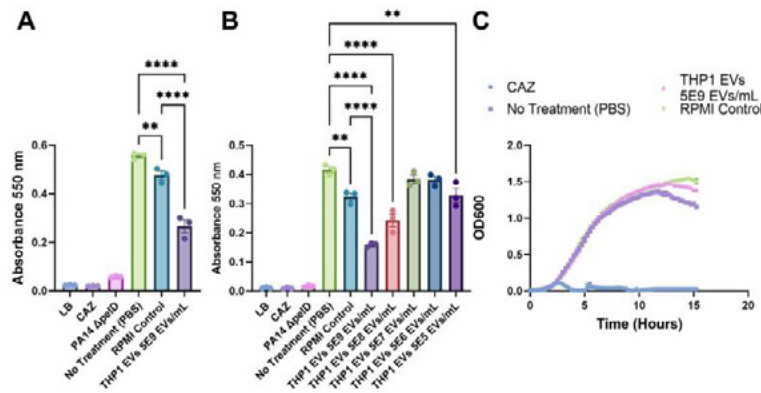
**Background** Antimicrobial resistance is implicated annually in over 35,000 deaths in the US and 1.27 million deaths worldwide. Thus, the need for new antimicrobial approaches is urgent and clear. A particular pathogen of concern is multidrug resistant *Pseudomonas aeruginosa* (PA), which is responsible for 32,000+ infections and 2,700+ deaths in the US yearly. One potential class of novel therapeutics is extracellular vesicles (EVs), which have been found to have intrinsic antimicrobial and anti-virulence properties. Additionally, EVs can provide a platform for combination antimicrobial therapy.

**Methods** Here, the antimicrobial activity of EVs on PA was explored in the context of biofilms of hypervirulent strain PA14. Preliminary studies identified human monocyte cell line THP-1 as a promising source of EVs for this application. THP-1 EVs were isolated using differential centrifugation and tangential flow filtration and analyzed using nanoparticle tracking analysis to determine size and concentration. These EVs were incubated with PA14 cells in a microtiter plate for 24 hours to allow biofilm formation. Then the biofilm was washed and stained with crystal violet followed by solubilization in 30% acetic acid and quantification with spectrophotometry to assess biofilm formation in the presence of THP-1 EVs.

**Results** THP-1 EVs were shown to reduce PA14 biofilm formation compared to negative controls no treatment (PBS) control and RPMI media control to account for the effects of co-isolated media contaminants (Fig. 1A) and this was shown to be dose dependent (Fig. 1B). Additionally, in a microtiter growth assay, THP-1 EVs were not shown to decrease PA14 growth, indicating an anti-virulence effect (Fig. 1C).

**Conclusions** Overall, these data implicate THP1 EVs as a potential antibiofilm therapeutic of interest, with mechanistic investigations still ongoing.

**Abstract Body:**



**Figure 1. THP-1 EVs inhibit PA14 biofilm formation (A)** A crystal violet-stained microtiter dish biofilm formation assay was performed, with positive controls: LB broth with no bacteria (LB), ceftazidime (CAZ), and PA14  $\Delta\text{pelD}$ , which cannot form biofilms in this *in vitro* model due to lack of the *pelD* exopolysaccharide. Negative control/no treatment was PA14 mixed with PBS. RPMI control is volume matched RPMI that undergoes the EV isolation procedure to account for media components that are co-isolated. (B) In the same assay, THP-1 EVs decreased biofilm formation in a dose dependent manner. (C) In a microtiter dish growth curve assay, THP-1 EV treatments were not shown to affect PA14 growth.

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**Control Number:** 2023-A-69-ASM-ESC  
**Session Title:** **Poster Presentations Session II**  
**Publishing Title:** **Time-Kill Kinetics of the Novel Broad Spectrum  $\beta$ -lactamase Inhibitor APC247**

**Author Block:**

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**Background** β-lactamase production represents the most common antimicrobial resistance mechanism in Gram-negative bacteria. Several β-lactamase families have been identified and can coexist, leading to multidrug resistance and difficult to treat infections caused by bacteria harboring these enzymes. Here we describe the *in vitro* interaction between meropenem and APC247, a novel broad-spectrum inhibitor targeting both metallo- and serine-β-lactamase enzymes, against multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates.

**Methods** *E. coli* IHMA997800 and *K. pneumoniae* AMA2650 were selected based on the expression of MBL and/or SBL enzymes and a high meropenem MIC of 256 and 128 mg/L, respectively. Checkerboards were conducted to evaluate the interaction between meropenem and APC247. Furthermore, time-kill curves were investigated for both strains with different concentrations of meropenem alone and in combination with APC247, as appropriate.

**Abstract Body:**

**Results** *E. coli* IHMA997800 and *K. pneumoniae* AMA2650 isolates were rendered meropenem sensitive by combination with APC247. In checkerboard assays, it was observed that meropenem MIC was reduced to breakpoint level (2 mg/L) by APC247 at 64 mg/L and 1 mg/mL, respectively. Combination time-kill assays revealed that APC247 restores meropenem susceptibility, leading to bacterial growth reduction by at least 2 log<sub>10</sub> CFU/mL after 3h exposure to 8 mg/L APC247 for *E. coli* IHMA997800 and 1 mg/mL for *K. pneumoniae* AMA2650. For the initial net growth rate of *E. coli* IHMA997800, the concentration of APC247 clearly had a pronounced influence on the size of Emax as well as EC50 of meropenem's apparent sigmoid effect curve.

**Conclusion** It was shown that APC247 restores meropenem susceptibility in multi-drug resistant clinical isolates *in vitro*. Results indicate that different concentrations of APC247 are needed for different isolates that may be related to the β-lactamase enzymes expressed by the isolates. Similar results were observed for multiple *E. coli* and *K. pneumoniae* clinical isolates.

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**Control Number:**

2023-A-71-ASM-ESC

**Session Title:**

**Poster Presentations Session II**

**Publishing Title:**

**Etrasimod and its Derivatives as Antibacterial Agents Against Gram-positive Bacteria**

**Author Block:**

M. Zore, P. San Martin Galindo, I. Reigada, K. Savijoki, A. Fallarero, J. Z. Patel, J. Yli-Kauhaluoma; Faculty of Pharmacy, Univ. of Helsinki, Helsinki, Finland

**Background:** Conventional discovery of antibiotics is a long and expensive process, with a low success rate, and has not been able to cope with the emergence of antibiotic resistance. (1) Over the past decades, drug repurposing has received increased attention as an attractive strategy for more efficient drug discovery, including antimicrobials. (2) We previously described the screening of FDA-approved drugs which led to identification of fingolimod, an S1PR modulator approved for the treatment of multiple sclerosis, as an antibacterial and anti-biofilm compound against *Staphylococcus aureus* (MIC 15 μM; 4.6 μg/mL) and *Acinetobacter baumannii* (MIC 25 μM; 7.7 μg/mL). (3) Thus, we set out to further explore the potential of other S1PR modulators as antibacterial agents.

**Methods:** Chemical synthesis and characterisation, antibacterial testing (MIC/MBC determination, time-kill, resistance development and checkerboard assay), cytotoxicity.

**Abstract Body:**

**Results:** We screened a small library of S1PR modulators against bacteria and identified etrasimod as a promising antibacterial compound. (4) Etrasimod, an investigational drug for the treatment of ulcerative colitis, displayed a potent activity against several Gram-positive bacteria, including drug-resistant *S. aureus* strains, *S. epidermidis* and *Enterococcus faecalis*, with an MIC 5 - 10 μM (2.3 - 4.6 μg/mL). It also displayed a synergistic effect with gentamicin against *S. aureus* and no *in vitro* toxicity towards mammalian cells. However, bacteria started to develop resistance after 10 days of exposure, which could potentially limit its use as antibacterial compounds. (4) We then prepared 32 etrasimod derivatives which provided insights on structure-activity relationships, and we found several etrasimod derivatives with more potent antibacterial activity. Indole derivative **MZII-182** showed the most potent antibacterial activity, with MIC 2.5 - 5 μM (1 - 2 μg/mL) against MRSA, *S. epidermidis*, *E. faecalis* and vancomycin-resistant *E. faecium*. Furthermore, it showed bactericidal mode of action, low toxicity against mammalian cells and low development of bacterial resistance. (unpublished data)

**Conclusions:** This study highlights the potential of etrasimod and its derivatives as novel antibacterial compounds against Gram-positive bacteria. 1) *Antibiotics* 2019, 8 (2), 45. 2) *Nat. Microbiol.* 2019, 4 (4), 565-577. 3) *Microorganisms* 2020, 8 (11). 4) *Front. Microbiol.* 2022, 13.

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**Control Number:**

2023-A-79-ASM-ESC

**Session Title:**

**Poster Presentations Session II**

**Publishing Title:** Discovery and Development of Novel Inhibitors for Bacterial Ribosomal Rna Synthesis as First-In-Class Antimicrobial

**Author Block:** Candidates

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**Background:** There is an urgent need for the discovery of new antibiotics with a new mode of action as antimicrobial resistance (AMR) had been one of the major threats to the global public health. Bacteria transcription is a validated but underutilized target for antimicrobial discovery. RNA polymerase (RNAP) is the core enzyme and a number of transcription factors are involved in regulation of the process in bacteria.

**Methods:** Our group discovered the first-in-class bacterial rRNA transcription inhibitor as a novel antimicrobial agent. NusB and NusE are essential transcription factors responsible for bacterial rRNA synthesis. Based on the highly conserved interface between NusB and NusE, a pharmacophore model was constructed for *in-silico* screening. We found one lead compound MC4 have antimicrobial activity. Optimization of the hit compound was carried out by target based *de novo* design and over 200 analogues were made to determine the structure-activity relationship.

**Abstract Body:**

**Results:** Some derivatives showed drastically improved antimicrobial activity against a panel of clinical antibiotic resistant strains including MRSA and VRSA with the minimal inhibitory concentration (MIC) comparable to marketed antibiotics. The mechanism of MC4 derivatives were examined. At the molecular level, representative MC4 derivatives inhibited NusB-NusE interaction, and the *in vitro* transcription. At the cellular level, MC4 is able to disrupt the subcellular localisation of the fluorescently labeled transcription machinery and reduce the total RNA and major rRNA. Since resistance in bacteria is a significant concern for antibiotic drugs, the possibility of resistance development was tested. Serial passaging of MRSA in the presence of representative MC4 derivatives at sub-inhibitory concentrations for 30 days did not generate resistant isolates compared to rifampicin control drug which generated 1000-fold of resistance after 5 days (unpublished). Representative MC4 derivatives showed very low level of haemolytic property against human blood cells, and high apparent permeability using the human intestinal epithelial cell line Caco-2. The MC4 derivatives were proven effective using a MRSA LD<sub>90</sub> sepsis mouse infection model, and the PK profiles and tissue distribution were also established (unpublished).

**Conclusions:** In summary, we developed first-in-class drug candidates protected with international patent. The preclinical data indicated the compounds have high potential for further development as an antimicrobial drug candidate.

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**Control Number:** 2023-A-81-ASM-ESC

**Session Title:** Poster Presentations Session II

**Publishing Title:** Susceptibility of the Anaerobes *Bacteroides* spp., *Clostridium* spp., and *Clostridioides difficile* to a Unique Synergistic Antibacterial Combination

**Author Block:** J. L. Pace<sup>1</sup>, P. Martinez Ponce<sup>2</sup>, E. Rios-Nieves<sup>3</sup>, L. Dubreuil<sup>4</sup>, T. Hartsell<sup>5</sup>, S. Gardlik<sup>6</sup>, S. Volla<sup>7</sup>; <sup>1</sup>FleurirABX, Elizabeth City, NC, NC, <sup>2</sup>FleurirABX, LLC, Elizabeth City, NC, <sup>3</sup>FleurirABX, Elizabeth City, NC, <sup>4</sup>Univ. of Lille, Lille, France, <sup>5</sup>Sentara Hlth.care, Elizabeth City, NC, NC, <sup>6</sup>FleurirABX, Raleigh, NC, <sup>7</sup>FleurirABX, Philadelphia, PA

**Background:** *Bacteroides* spp. and *Clostridium* spp. are encountered as a principle cause or in mixed moderate to severe infections of various soft tissues. Additionally, severe life-threatening colitis due to *Clostridioides difficile* remains a bane of modern medical practice. Where carbapenem or glycopeptide resistance is expected, antibiotic combinations are typically utilized. Unexpectedly, it has been observed that a combination (FTS) of fosfomycin (F) and trimethoprim-sulfamethoxazole (TS) exerts in-vitro antibacterial synergy across these anaerobic species.

**Methods:** A checkerboard version of the CLSI Agar Dilution method with Glucose-6-phosphate supplemented Brucella Blood Agar was used. Minimal inhibitory concentrations (MIC) were determined for FTS, F or TS alone, and other antibiotic comparators. Incubation was in an anaerobic GasPak EZ container system at 35°C for 48 hours before visual observation of bacterial growth. The MICs were reported as the lowest drug concentrations inhibiting visible growth.

**Abstract Body:**

**Results:** *Bacteroides fragilis* and *B. ovatus* were resistant to F alone (MIC >128 mg/ml) based on extrapolated breakpoints. *Clostridium perfringens*, *C. septicum*, and *Clostridioides difficile* were susceptible to F alone (MIC <32mg/L). All bacterial isolates of any species evaluated were resistant to TS alone (MIC 4/76 - >32/>608 mg/L). Unexpectedly nearly all isolates were susceptible to the FTS combination (MIC <32/<38 mg/L) with antibacterial synergy (FIC <0.5) achieved against all, with the exception of two *C. perfringens* isolates which were susceptible to the F component (1-8 mg/L) but resistant to the TS component (MIC 4/76 - 8/152 mg/L) when in combination even though MIC synergy was achieved.

**Conclusions:** Resistance to any other antibacterial class did not impact the unique synergistic activity of the FTS combination against anaerobes. FTS may possess an adequate spectrum for the antibacterial therapy of many mixed and anaerobic infections, and merits clinical evaluation.

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**Control Number:** 2023-A-84-ASM-ESC

Session  
Title: **Poster Presentations Session I**

Publishing  
Title: **Burden of Antimicrobial Resistance in Hospitalized Patients with Cancer: A Multicenter Analysis**

Author  
Block: V. Gupta, K. Yu, C. Sheets, **D. Flayhart**, Cancer & AMR Consortium; Becton, Dickinson & Co., Franklin Lakes, NJ

**Background:** Infections are the second leading cause of death in patients with cancer, there has not been any large-scale assessments of AMR in the cancer population. This multi-center study compared the prevalence/incidence of pathogens isolated and related drug resistance in hospitalized cancer and non-cancer patients in the US. **Methods:** Adult patients ( $\geq 18$  years) with 30-day nonduplicate isolates from hospital settings from 4/2018 - 12/2022 were evaluated across 231 facilities in the BD Insights Research Database. Prevalence and incidence for antimicrobial resistance (AMR) types in non-contaminant gram-negative, gram-positive bacteria, and fungal pathogen incidence across all culture sources were evaluated. Cancer was defined as patients that were prescribed cancer medications in the prior 365 days (<https://www.cancer.gov/about-cancer/treatment/drugs>) from an index event or were hospitalized to a cancer unit. All other admissions were categorized as non-cancer. **Results:** Across 5,207,336 admissions, 6% (314,201) were categorized as cancer and 94% (4,893,135) as non-cancer. The rate of pathogen identification was higher in cancer patients across all pathogen groups evaluated. The prevalence (% NS) was significantly lower in cancer vs. non-cancer patients for most AMR types evaluated, but incidence (rate/1000 adm) was significantly higher for all AMR types in cancer vs. non-cancer patients (Table). The incidence of VRE and fungal pathogens was ~2-fold higher and AMR in gram-negative pathogens was ~1.5-fold higher in cancer than non-cancer patients. **Conclusions:** Hospitalized cancer patients have a higher incidence (/1000 adm) of AMR than non-cancer patients. Appropriate diagnostics and local AMR incidence studies could inform optimal use of newer or specialized antimicrobials when treating serious infections in cancer patients.

Abstract  
Body:

**Table 1. Prevalence (% NS) and incidence (/1000 admissions) of antimicrobial resistance and pathogen distribution in hospitalized patients with and without cancer.**

Pathogen and AMR Type	Non-Cancer (N=4,893,135 Admissions)		Cancer (N=314,201 Admissions)		Overall Total (N=5,207,336 Admissions)	
	% NS (n/N)	/1000 Adm	% NS (n/N)	/1000 Adm	% NS (n/N)	/1000 Adm
<b><i>P. aeruginosa</i> Total Isolates (N)</b>	<b>49,542</b>	<b>10.12</b>	<b>5,875</b>	<b>18.7</b>	<b>55,417</b>	<b>10.64</b>
Quinolone (FQ) NS	21.0% (10,428)	2.13	17.2% (1,010)*	3.21*	20.6% (11,438)	2.2
Multidrug resistant (MDR)	9.4% (4,663)	0.95	7.3% (431)*	1.37*	9.2% (5,094)	0.98
Carbapenem NS (Carb-NS)	11.7% (5,798)	1.18	9.6% (566)*	1.8*	11.5% (6,364)	1.22
<b>Enterobacteriales (ENT) Isolates (N)</b>	<b>310,946</b>	<b>63.55</b>	<b>28,511</b>	<b>90.74</b>	<b>339,457</b>	<b>65.19</b>
Quinolone (FQ) NS	24.2% (75,218)	15.37	24.3% (6,925)	22.04*	24.2% (82,143)	15.77
Multidrug resistant (MDR)	6.4% (19,776)	4.04	6.9% (1,970)*	6.27*	6.4% (21,746)	4.18
Carbapenem NS (Carb-NS)	1.4% (4,425)	0.9	1.5% (437)	1.39*	1.4% (4,862)	0.93
<b>ENT Isolates Tested for ESBL (N)</b>	<b>270,622</b>	<b>55.31</b>	<b>24,353</b>	<b>77.51</b>	<b>294,975</b>	<b>56.65</b>
ESBL + cases (n)	13.4% (36,293)	7.42	15.0% (3,647)*	11.61*	13.5% (39,940)	7.67
<b>Enterococcus spp. Isolates (N)</b>	<b>66,207</b>	<b>13.49</b>	<b>7,268</b>	<b>23.13</b>	<b>73,295</b>	<b>14.08</b>
Vancomycin resistant enterococcus (VRE)	14.6% (9,633)	1.97	17.0% (1,237)*	3.94*	14.8% (10,870)	2.09
<b><i>S. aureus</i> Isolates (N)</b>	<b>103,015</b>	<b>21.05</b>	<b>8,154</b>	<b>25.95</b>	<b>111,169</b>	<b>21.35</b>
Methicillin resistant <i>S. aureus</i> (MRSA)	46.7% (48,110)	9.83	42.9% (3,497)*	11.13*	46.4% (51,607)	9.91
<b>Fungal/Yeast Isolates (N)</b>	<b>41,755</b>	<b>8.53</b>	<b>5,864</b>	<b>18.7*</b>	<b>47,619</b>	<b>9.14</b>
<i>Candida albicans</i>	25,445	5.20	3,468	11.0*	28,913	5.55
<i>Candida (Torulopsis) glabrata</i>	5,580	1.14	751	2.39*	6,331	1.22
All others	10,730	2.19	1,645	5.31*	12,375	2.37

\* P < 0.001 for Cancer vs. Non-cancer; Adm, admissions; NS, non-susceptible; ESBL, Extended spectrum beta-lactamase; spp. species

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Control  
Number: 2023-A-89-ASM-ESC

Session  
Title: **Poster Presentations Session II**

Publishing  
Title: **Predicting Efficacious Exposures for a Novel  $\beta$ -lactam/ $\beta$ -lactamase Inhibitor with a Semi-mechanistic Model**

Author  
Block: **H. R. Meredith**<sup>1</sup>, W. Hope<sup>2</sup>, S. Ripp<sup>3</sup>, L. McEntee<sup>2</sup>, A. Johnson<sup>2</sup>, G. Stone<sup>3</sup>, S. Das<sup>2</sup>, B. Boras<sup>4</sup>; <sup>1</sup>Pfizer, Cambridge, MA, <sup>2</sup>Univ. of Liverpool, Liverpool, United Kingdom, <sup>3</sup>Pfizer, Groton, CT, <sup>4</sup>Pfizer, La Jolla, CA

Abstract  
Body: **Background:** Optimizing antibiotic regimens is critical for successful treatment outcomes. To predict effective regimens, mathematical models have been used to relate treatment outcome and pharmacokinetic/pharmacodynamic (PKPD) properties derived from preclinical *in vivo* studies. This relationship is often established with an Emax model; however, it relies on indices that

do not capture the temporal dynamics of PKPD processes and are often defined by the minimum inhibitory concentration (MIC), which may not have good *in vitro-in vivo* correlation (IVIVC). A promising alternative is the semi-mechanistic model that captures temporal dynamics and can be adjusted for the MIC's IVIVC.

**Methods:** Here we use ceftibuten-avibactam (CTB-AVI), a novel oral  $\beta$ -lactam/ $\beta$ -lactamase inhibitor (BL/BLI), to compare the Emax and semi-mechanistic models. A mouse thigh infection model generated PKPD data for 7 *Enterobacteriales* isolates with a range of CTB MICs. Both models were fit to data and used to estimate combinations of CTB-AVI exposures that could achieve bacterial stasis after 24 hours of treatment, the target outcome.

**Results:** For different regimens and isolates, the Emax model captured the change in bacterial burden after treatment while the semi-mechanistic model captured the temporal dynamics of the bacterial antibiotic response. With decreasing CTB exposure, both models estimated increasing AVI exposure was necessary for bacterial stasis. While the AVI exposure estimates from both models converged at high CTB exposures, they diverged at lower CTB exposures with the semi-mechanistic model estimating more AVI. Ultimately, a combination of CTB and AVI exposure was selected that both models agreed upon.

**Conclusions:** While semi-mechanistic models have been used for decades, they are not widely used in regulatory filing as a tool to set PKPD targets and estimate efficacious doses. By comparing the modeling approaches and their recommended exposures for this novel BL/BLI, we aim to provide a case study in how semi-mechanistic models can be a better tool for informing the dose selection process both for internal decision making and discussions with regulators.

**Control Number:** 2023-A-90-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **Efficacy of an AMP-Based Therapy to Control Bovine Mastitis Pathogens**

**Author Block:** H. Mantovani<sup>1</sup>, A. Moreira<sup>1</sup>, K. Domingues<sup>2</sup>, A. Assumpcao<sup>1</sup>, K. Camargo<sup>1</sup>, N. Aulik<sup>1</sup>; <sup>1</sup>Univ. OF WISCONSIN-MADISON, Madison, WI, <sup>2</sup>Univ. Federal de Viçosa, Vicoso, Brazil

**Background:** Bovine mastitis is the costliest disease in the dairy sector and is the main cause for antibiotic use in dairy cattle. Antimicrobial peptides (AMPs) have been proposed as alternatives to antibiotics to control bovine mastitis pathogens. In this study, the efficacy of AMPs and chemical adjuvants was assessed against a range of mastitis-causing pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus chromogenes*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*.

**Methods:** Five AMPs identified in the rumen and human gut microbiomes were evaluated, along with two potential chemical adjuvants. Minimum inhibitory concentrations (MICs) were determined for each bacterial strain (n=35). The two most effective treatments with a broad spectrum of activity were selected for combinatorial assays. The Fractional Inhibitory Concentrations Index (FICI) was calculated to determine interaction effect. A FICI  $\leq 0.5$  was indicative of synergism. The lowest FICI combination, which resulted in inhibition of all 35 strains tested, was chosen for further evaluation. Time-dependent killing assays were performed to assess bactericidal efficacy of the combination. Cytotoxicity against bovine mammary alveolar (MAC-T) cells was evaluated using the MTT assay. Hemolytic activity was assessed against fresh bovine erythrocytes.

**Abstract Body:**

**Results:** AMP1 and CA1 exhibited the highest antimicrobial activity, reducing the final optical density at 600 nm (OD600) by 95% and 86.8%, respectively, and were therefore selected for further analyses. FICI values ranged from 0.1 to 0.5, indicating synergism. The combination of AMP1 (32  $\mu$ g/mL) and CA1 (1,024  $\mu$ g/mL) exhibited superior antimicrobial activity against all bacterial strains, at significantly lower concentrations (4 and 16-fold reduction for AMP1 and CA1, respectively) than each compound individually. AMP1+CA1 combination reduced the viable population by 1,000-fold within 12 hours. The combination is non-hemolytic in concentrations up to 16-fold higher than the established MIC values (P = 0.41). However, cytotoxic effects were observed for CA1 against MAC-T cells (P = 0.01).

**Conclusions:** These results indicate that AMP1 can be used to develop antibiotic-free formulations to control contagious and environmental mastitis pathogens. Further experiments will focus on developing delivery systems to evaluate the efficacy of these molecules *in vivo*.

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**Control Number:** 2023-A-92-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **Observations of Bacterial Persister Population Dynamics in Antibiotic Environments Using a Library of *Escherichia coli* Gene Deletion Strains**

**Author Block:** A. Muto<sup>1</sup>, S. Hingley-Wilson<sup>2</sup>, C. Furusawa<sup>1</sup>, J. McFadden<sup>2</sup>, H. Mori<sup>3</sup>; <sup>1</sup>RIKEN, Suita, Japan, <sup>2</sup>Univ. of Surrey, Guildford, United Kingdom, <sup>3</sup>Guangzhou, 44, China

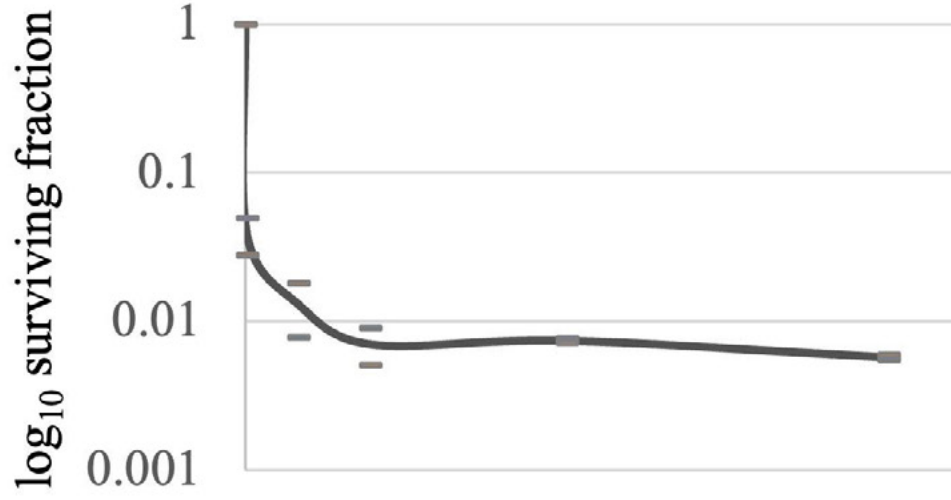
**Background:** Bacterial persistence is a phenomenon in which a small fraction of a clonal bacterial population can survive lethal conditions. Persistence under antibiotic treatment is a possible cause of extended treatment and increased severity of infectious diseases. Recently, evidence suggesting that persistence enhances resistance acquisition has been reported, and the importance of the exploration of the persistence mechanism is increasing.

**Methods:** An *E. coli* knock-out library carrying artificial nucleotide sequences(DNA bar-code) in their chromosomes were used. By amplification and sequencing of the bar-code region, we have obtained frequency information of strains in the samples and observed time-course dynamics changes. For the candidate strains obtained, single-cell observation and image tracking were performed.

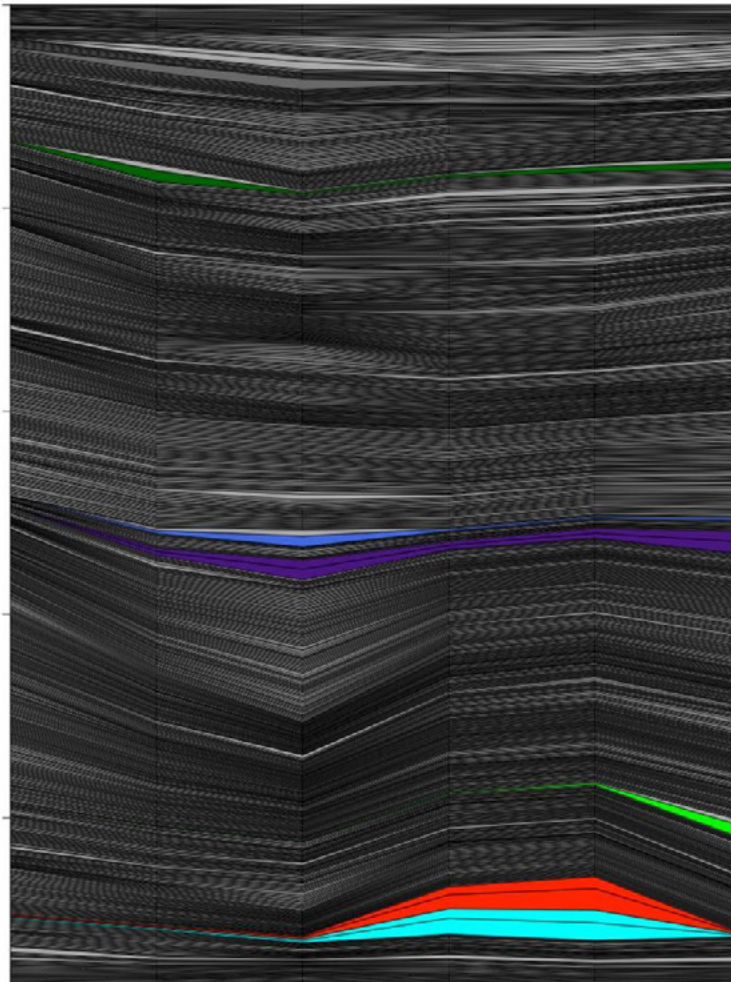
**Results:** We have observed the population dynamics change in the persister colonies grown on antibiotics-free agar plates after spreading an antibiotics-treated mixed culture of mutant strains(Fig). From the observation, two mutant strains with high-persister phenotypes were identified and individually confirmed. Microfluidics experiments using confocal microscopy showed a high regrow rate of the strains after the removal of antibiotics and persister cells.

**Conclusions:** We have obtained a new observation of the bacterial persister population dynamics. From the observation, we found the new high-persister genotype and observed the phenotype at the single-cell level. Since the genes deleted in the strains are known to contribute to bacterial resistance, our observations may contribute to the elucidation of mechanisms related to the relationship between resistance and persistence.

# Ciprofloxacin



Relative abundance of each knockout strain  
in persister colonies



Session  
Title: **Poster Presentations Session II**

Publishing  
Title: **Pharmacokinetics-pharmacodynamics (PK-PD) of new broad-spectrum agent BWC0977**

Author  
Block: **L. McEntee<sup>1</sup>, N. Farrington<sup>1</sup>, A. Johnson<sup>1</sup>, I. Horner<sup>2</sup>, A. Stevenson<sup>2</sup>, J. Unsworth<sup>1</sup>, H. Katakonda<sup>3</sup>, B. Subramanian<sup>4</sup>, A-G. Martson<sup>1</sup>, S. Das<sup>1</sup>; <sup>1</sup>Univ. of Liverpool, Liverpool, United Kingdom, <sup>2</sup>Liverpool, United Kingdom, <sup>3</sup>Bugworks, Bangalore, India, <sup>4</sup>Bangalore, India**

**PK-PD of new broad-spectrum agent BWC0977**<sup>1</sup>L McEntee, <sup>1</sup>N Farrington, <sup>1</sup>A Johnson, <sup>1</sup>I Horner, <sup>1</sup>A Stevenson, <sup>1</sup>J Unsworth, <sup>2</sup>H Katakonda, <sup>2</sup>B Subramanian, <sup>1</sup>S Das, <sup>1</sup>A-G Martson<sup>1</sup>APT Group, University of Liverpool, UK <sup>2</sup>Bugworks, Bangalore, India **Background:** New antibacterial agents for drug-resistant infections are urgently needed. BWC0977 is a novel potent gyrase-topoisomerase inhibitor with activity against ESBLs, AmpC, KPCs and fluoroquinolone-resistant strains currently being assessed in a Phase 1 clinical programme. Herein we describe the PK-PD of BWC0977 assessed in the murine thigh infection model. **Methods:** A 26-hour neutropenic murine thigh infection model was used for dose ranging and fractionation. Challenge strain *P. aeruginosa* NCTC 13921 was used for dose fractionation studies and PK and further isolates of *P. aeruginosa*, *A. baumannii*, and Enterobacterales with resistance mechanisms were used for dose ranging (**Table 1**). A destructive design was employed, with mice dosed subcutaneously 2 hours post-infection and bacterial density (CFU/g) infection compared to a 2h baseline control taken as the endpoint. A total daily dose of BWC0977 160 mg/kg was fractionated; 160 mg/kg q24h, 80 mg/kg q12h, 40 mg/kg q6h with PD samples taken at 2, 8, 14, 26 hours post-infection. For dose ranging, BWC0977 was administered q8h and polymyxin B was used as comparator. For PK, BWC0977 was dosed at 10, 40, 80, 120 mg/kg q24h with samples taken at 0.5, 1, 2, 4, 6, 8, 24 hours post-dose. **Results:** A 2-compartment model with linear clearance was fitted to the PK data. The more fractionated regimens did not outperform the once daily regimen. The best PK-PD index was defined to be *f*AUC/MIC. There was an excellent dose response to the tested bacterial isolates. *K. pneumoniae* and *A. baumannii* strains showed the highest variability in the CFU decline. **Conclusions:** The defined PK/PD index for efficacy for BWC0977 is *f*AUC/MIC.

**Table 1.** Challenge strains

Abstract  
Body:

Species	Strain	Molecular info	BWC0977 MIC (mg/L)	Meropenem MIC (mg/L)
<i>P. aeruginosa</i>	ATCC 27853	WT	0.5	0.25-0.5
<i>P. aeruginosa</i>	NCTC 13921	SPM-1	0.25	>64
<i>P. aeruginosa</i>	NCTC 13437	VIM-10, VEB-1	0.5	>64
<i>A. baumannii</i>	NCTC 13301	OXA-23	0.25	>64
<i>A. baumannii</i>	ATCC 17978	WT	0.125-0.25	0.25-1
<i>K. pneumoniae</i>	NCTC 13465	CTX-M25	0.125-0.25	0.125
<i>K. pneumoniae</i>	ATCC 43816	WT	0.25	
<i>E. coli</i>	ATCC BAA-2523	OXA-48	0.06-0.125	0.5-1
<i>E. coli</i>	NCTC 13462	CTX-M2	0.06	0.5-1

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Control  
Number: 2023-A-98-ASM-ESC

Session  
Title: **Poster Presentations Session II**

Publishing  
Title: **Murine pharmacodynamics of SPR206**

Author  
Block: **L. McEntee**<sup>1</sup>, A. Johnson<sup>1</sup>, N. Farrington<sup>1</sup>, I. Horner<sup>1</sup>, A. Stevenson<sup>1</sup>, J. Unsworth<sup>1</sup>, B. Kane<sup>2</sup>, N. Cotroneo<sup>2</sup>, I. Critchley<sup>2</sup>, J. Bruss<sup>3</sup>, S. Das<sup>1</sup>; <sup>1</sup>Univ. of Liverpool, Liverpool, United Kingdom, <sup>2</sup>Spero Therapeutics, Cambridge, MA, <sup>3</sup>Alarus Dev. Intl., LLC, Pagosa Springs, CO

**Background:** SPR206 is a polymyxin derivative with improved efficacy and reduced toxicity relative to polymyxin B in preclinical models. SPR206 is in development for nosocomial pneumonia and has a broad spectrum of activity against Gram-negative pathogens, including carbapenem-resistant *A. baumannii* (CRAB), *P. aeruginosa* (CRPA), and *K. pneumoniae*. *In vivo* murine models of infection were used to identify the pharmacokinetic-pharmacodynamic (PK-PD) index that describes the link between antibacterial effect and drug exposure. This index was used to estimate exposure targets required for achieving stasis and logarithmic killing, with focus on *P. aeruginosa* and *K. pneumoniae*. **Methods:** Dose finding and dose fractionation studies were performed in neutropenic CD-1 mouse thigh and lung models of infection against wild-type and non-wild-type *P. aeruginosa* and *K. pneumoniae*. The study endpoint was the bacterial density (CFU/g) in the thigh or lung, 26 hours post infection. SPR206 was subcutaneously administered 2 hours post infection. Plasma and ELF PK were established using dosages of 5, 25, and 50 mg/kg q8h, with drug concentrations measured in the 1st and 3rd dosing intervals by LC/MS/MS, and a population PK model was fitted to the data in Pmetrics. A traditional dose fractionation study was performed by fractionating doses of SPR206 25, 50, 65, and 75 mg/kg q6h, q12h, and q24h and the Sigmoid Emax model was used to fit the data to different PK-PD indices. *P. aeruginosa* and *K. pneumoniae* were co-modelled by species to determine median exposure targets. **Results:** In Pmetrics, a 4-compartment structural model was fitted to the plasma and ELF data. SPR206 showed approximately 62% partitioning into the lungs for all dose groups. SPR206 was concentration-dependent with a long persistent effect. Although AUC:MIC and Cmax:MIC fitted the data well, the totality of the data generated was considered and AUC:MIC was selected, which has also been well established for the polymyxin class. For *P. aeruginosa*, fAUC:MIC plasma targets in the thigh were 39-85 and in the lung 44-108, for stasis and 2 log kill, respectively. AUC:MIC ELF targets in the lung were 33-84 for stasis and up to 2 log kill. For *K. pneumoniae*, fAUC:MIC plasma targets in the lung were 582-1044 for stasis and up to 2 log kill, in the thigh the stasis target was 220, and AUC:MIC ELF targets in the lung were 448-598 for stasis and 1 log kill. **Conclusions:** The exposure targets derived from these studies will be used to support probability of target attainment (PTA) in patients who will receive SPR206.

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Control  
Number: 2023-A-106-ASM-ESC

Session  
Title: **Poster Presentations Session II**

Publishing  
Title: **Optimization of *Pseudomonas aeruginosa* LasB Inhibitors Guided by *In Vitro* ADMET Profiling**

Author  
Block: **A. M. Kany**<sup>1</sup>, J. Konstantinovic<sup>1</sup>, A. Alhayek<sup>1</sup>, A. Klein<sup>1</sup>, D. Kolling<sup>1</sup>, R. Shafiei<sup>1</sup>, C. Schütz<sup>1</sup>, B. Loretz<sup>1</sup>, C-M. Lehr<sup>1</sup>, K. Rox<sup>2</sup>, J. Hauptenthal<sup>1</sup>, A. K. H. Hirsch<sup>1</sup>; <sup>1</sup>Helmholtz Inst. for Pharmaceutical Res. Saarland, Saarbrücken, Germany, <sup>2</sup>Helmholtz Ctr. for Infection Res., Braunschweig, Germany

**Background:** Infections caused by *Pseudomonas aeruginosa* are becoming increasingly difficult to treat due to the rise of antimicrobial resistance. This poses a particular threat to patients suffering from *e.g.* hospital-acquired or ventilator-associated pneumonia (HAP/VAP), cystic fibrosis or non-cystic fibrosis bronchiectasis. To develop novel, non-traditional treatments targeting *P. aeruginosa* virulence, the secreted protease elastase (LasB) represents a prime target due to its key role in bacterial virulence and its extracellular localization. We optimized a thiol-based LasB inhibitor scaffold by exploring different zinc-binding groups aiming at improved *in vitro* activity and a favorable *in vitro* ADMET profile. **Methods:** Alongside *in vitro/ex vivo* activity profiling of LasB inhibitors, we investigated their *in vitro* ADMET properties using a tiered screening cascade. The established models assess physicochemical properties, cytotoxicity, metabolic and plasma stability as well as plasma protein binding for various species. We further investigated cell permeability across Calu-3 monolayers, which provides essential information regarding lung retention, which is required for *in vivo* efficacy. **Results:** Following the multiparameter approach described above, we have identified a phosphonate-based inhibitor scaffold as most promising to be advanced into more detailed *in vivo* profiling. The determined *in vitro* ADMET properties could be translated into favorable bioavailability and efficacy in murine *in vivo* infection models. **Conclusions:** Rational screening of *in vitro* ADMET parameters of LasB inhibitors alongside their on-target activity provides essential information favoring the development of non-traditional treatment options for infections with drug-resistant *P. aeruginosa*. The generated information serves as a screening tool for further compound optimization. **Conflict of interest:** We declare that parts of the authors are co-inventors on an international patent application (PCT/EP2021/073381) that includes technologies described in this abstract.

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**Control Number:** 2023-A-110-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **Synergistic Bactericidal Activity of Bovicin HC5 in Combination with Cephalosporins Against Bovine Mastitis Pathogens and MRSA**

**Author Block:** H. Mantovani<sup>1</sup>, K. Domingues<sup>2</sup>, L. Oyama<sup>3</sup>, S. Huws<sup>3</sup>; <sup>1</sup>Univ. OF WISCONSIN-MADISON, Madison, WI, <sup>2</sup>Univ. OF WISCONSIN-MADISON, Vicoso, Brazil, <sup>3</sup>Queen's Univ. Belfast, Belfast, United Kingdom

**Background:** Bovine mastitis is an inflammatory disease that represents a major economic challenge for dairy farmers. Mastitis is the costliest disease in the dairy industry and the leading cause of antibiotic use in dairy cattle. In this work, the effectiveness of combinations containing the lantibiotic bovicin HC5 and reduced concentration of antibiotics was demonstrated against the main etiologic agents of bovine mastitis in addition to antibiotic resistant strains and MRSA.

**Methods:** The synergism between ceftiofur (Ceft) or cephalixin (Cepha) with bovicin HC5 (HC5) was evaluated against *S. aureus* strains using dose-response matrices (checkerboard) for FICI determination. The killing kinetics was evaluated by time-kill assays and safety assessments were performed by hemolysis of bovine red blood cells and cytotoxicity (MTT) assays using primary bovine mammary alveolar cells (MAC-T). The impact on biofilm formation and disruption was also evaluated. The effects on membrane permeability of each antimicrobial or the combination was determined.

**Abstract Body:**

**Results:** The Ceft-HC5 and Cefa-HC5 combinations showed FICI of 0.53 and 0.58 respectively, with a reduction of up to 85% in the amount of antibiotic needed to inhibit the growth of target bacteria. Combinations of ceftiofur and cephalixin with bovicin HC5 increased the rate of killing compared to the antibiotics used alone. The MIC of a ceftiofur-resistant *S. uberis* strain was reduced from 256 µg/ml to 2 µg/ml when ceftiofur was combined with 5.6 µg/ml of bovicin HC5. The lantibiotic was capable to disrupt established bacterial biofilms and the combination showed synergism against MRSA with a FICI minimum < 0.35. When a concentration of bovicin HC5 ≤ 11.2 µg/ml was used, the MIC of ceftiofur decreased from ≥256 µg/ml to <2 µg/ml for MRSA. No major changes in membrane integrity were observed upon treatment with bovicin HC5 or the lantibiotic+antibiotic. The synergism of bovicin HC5 was maintained for other beta-lactams (penicillin and meropenem), suggesting complementarity of mechanisms of action. The USA300 strain, a clinically important MRSA, showed reduction in the MIC to meropenem from 6.6 µg/ml to 0.33 µg/ml when in combination with 11.2 µg/ml bovicin HC5. No increase in toxicity was observed for the combination.

**Conclusions:** The lantibiotic bovicin HC5 shows synergism with beta-lactam antibiotics and could be a therapeutic adjuvant to improve the efficacy of traditional antibiotics while reducing resistance.

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**Control Number:** 2023-A-114-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **Diagnostic Accuracy of Rapid Assays For Diagnosis of Bloodstream Infections And Antimicrobial Profiling of Pathogens That Causes Bloodstream Infections At a Tertiary Hospital In Polokwane, Limpopo Province, South Africa**

**Author Block:** N. A. Maswanganyi, R. M. Lekalaka-Mokaba, I. Rukasha, D. Mukavhanyedzi; Univ. OF LIMPOPO - SOUTH AFRICA, Polokwane, South Africa

**Background:** Antimicrobial resistance is a significant global public health threat. There is a high mortality and morbidity rate that is associated with multi-drug resistance pathogens in South Africa, which negatively impact the patients' clinical outcome. Turnaround time for diagnosis of BSI is long, rendering the results clinically irrelevant. Thus, there is a need for a faster and more accurate diagnosis platform for diagnosis of BSI. There is also a need to employ more ways to minimize antimicrobial resistance globally and to develop more antimicrobials that will be more effective against these MDR microorganisms. There is also a need

**Abstract Body:**

**Methods:** We conducted a prospective cross-sectional study in patients suspected of having Bloodstream infections at Pietersburg Hospital, Polokwane, South Africa from February to May 2023. The blood cultures were incubated in the BD Bactec machine. The positive blood cultures were run on the VITEK 2 and BioFire Film Array BCID2 machines, to identify the pathogens, their AMR genes, as well as their antimicrobial susceptibility patterns. The data was analysed using Excel. **Results:** Of the pathogens that were isolated from the positive blood cultures, most of them were Gram-negative bacilli. The most common organisms isolated were *Acinetobacter baumannii* complex constituting 30%, followed by *Enterobacter cloacae* complex with 24%, *Klebsiella pneumoniae* group with 18%, *Escherichia coli* with 15%, and *Pseudomonas aeruginosa* with 12%. The AMR genes identified were *mecA/C* gene (33%), CTX-M (33%), NDM (18%), OXA-48 Like (12%), and VIM (3%). Of all the antimicrobials tested, ampicillin (82%), cefotaxime (77%), and trimethoprim-sulfamethoxazole (76%), showed the highest level of resistance. Most pathogens were isolated from male patients (48%), and from children between 0 to 5 years (48%). **Conclusion:** There is a significant rise in the statistics of Carbapenem-resistant organisms. The current state of clinical care is severely hampered by antimicrobial resistance. The impact of multidrug-resistant organisms may be reduced in the future with the use of novel antimicrobial methods, modified usage of antimicrobial agents, and public health measures. The BCID2 provides a faster and more accurate diagnosis of BSI as compared to the traditional culture methods and VITEK2.

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**Control Number:** 2023-A-121-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **Nanocomposite of Halloysite Clay, Nano-silver, and Tannic Acid as a Therapy to Combat Multi-drug Resistant *Salmonella* Typhimurium in a *Caenorhabditis elegans* Model of Intestinal Infection**

**Author Block:** S. Majumder, C. Viau, A. Brar, J. Xia, S. George; McGill Univ., Sainte anne de bellevue, QC, Canada

**Abstract Body:** **Background** The prevalence of antimicrobial resistance (AMR) among pathogenic bacteria in livestock warrants alternate therapeutic strategies that are efficient in remediating zoonotic infections. Combination therapy with more than one antimicrobial with complementary action has shown possibilities to prevent or slow down AMR. The application of clay-based biomaterials could, however, resolve challenges of poor bioavailability, cytotoxicity, stability, release, and overdosing and play a significant role in formulating effective therapeutics. **Methods** In this study, a nanocomposite (GH-TA-Ag-NT) containing silver nanoparticles (Ag NPs) grafted onto tannic acid (TA)-modified halloysite nanotubes (hal NTs) was generated and compared with TA-stabilized Ag NPs (Ag-TA-NP) for physicochemical and antibacterial properties. The TEM, FT-IR, DSC, and XRD spectroscopies were used for physicochemical characterization, while fluorometric assays were used for quantitative analysis of antibacterial properties. The toxicity and antibacterial efficiency of GH-TA-Ag-NT to eliminate gastrointestinal infection were tested using *S. Typhimurium*-infected Caco-2 cells and the *Caenorhabditis elegans* model. **Results** GH-TA-Ag-NT was biocompatible as  $\leq 125 \mu\text{g/mL}$  showed no toxicity to human intestinal Caco-2 cells. It demonstrated enhanced stability and drug bioavailability with a slow release of  $\text{Ag}^+$  and TA. It showed a combinatorial effect with a synergistic antibacterial behavior mediated through anti-efflux, anti-biofilm properties, oxidative stress, loss of bacterial membrane potential, and integrity and, thus, showed superior antibacterial performance when tested against multi-drug resistant (MDR) *Salmonella enterica* serovar Typhimurium (isolated from swine). It also remediated intracellular infection of *Salmonella* in the Caco-2 cells. A  $62.5 \mu\text{g/mL}$  of GH-TA-Ag-NT was non-toxic, significantly reduced *Salmonella* colonization in *Caenorhabditis elegans*, and improved survivability. **Conclusion** We demonstrated a nano-enabled antibacterial combination therapy (NeACT) using GH-TA-Ag-NT with better antimicrobial and physicochemical properties and thus propose potential use as a therapeutic in animal agriculture.

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**Control Number:** 2023-A-131-ASM-ESC

**Session Title:** **Poster Presentations Session II**

**Publishing Title:** **Effect of Media on the Bactericidal Activity of NOSO502, a Novel Odilorhabin Antibiotic**

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**Abstract Body:** **Background:** NOSO-502 is a first in class peptide belonging to the Odilorhabdins. Odilorhabdins were discovered from the species *Xenorhabdus* and act by inhibiting bacterial translation which results in broad *in vitro* potency against Gram-negative pathogens including multi-drug resistant strains. It is known that peptones present in Muller Hinton Broth (MHB) can inhibit the antibacterial activity of NOSO502 in MIC tests affecting absolute MIC values and reproducibility. The impact of media type on NOSO502s bactericidal activity is unclear so we have explored this by use of a range of pharmacologically relevant NOSO502 concentrations and different test media. **Methods:** Time kill studies were performed over 48hrs using NOSO502 concentrations of 0, 5, 10, 15 and 30mg/L against *E. coli* ATCC 25922 at an inoculum of  $2 \times 10^6$  cfu/mL. Three media were selected: MHB at 100% and 50% concentrations, RPMI and DMEM. Viable counts were determined at over 48h in triplicate for each drug concentration-media combination and log change in viable count at 24h (d24) and area-under-the-bacterial-kill-curve after 24h (AUBKC) taken as the primary endpoint measures. **Results:** *E. coli* grew well in all the media reaching bacterial loads of  $4 \times 10^8$  cfu/ml after 4hrs incubation. At a concentration of 5mg/L NOSO502 the d24s were:  $+2.1 \pm 0.4$  in 100% MNB,  $-2.2 \pm 0.6$  in 50% MHB,  $-0.4 \pm 1.8$  in RPMI and  $-0.6 \pm 1.6$  in DMEM. At NOSO 502 concentrations of 10mg/L d24 were:  $-2.4 \pm 2.2$  in 100%MHB,  $-3.8 \pm 0.7$  in 50% MHB,  $-2.0 \pm 0.6$  in RPMI and  $-3.4 \pm 1.0$  in DMEM. At NOSO502 concentrations of 30mg/L d24 were  $-4.1 \pm 0.2$  100%MHB,  $-4.2 \pm 0.1$  50% MHB,  $-3.6 \pm 0.8$  RPMI and  $-4.2 \pm 0.2$  DMEM. Similar trends were apparent with AUBKC with 100% MHB producing the largest areas (least killing) and 50%MHB, RPMI and DMEM lowest areas (most killing). ANOVA indicated no significant difference between killing in 50%MHB, RPMI and DMEM but for NOSO502 concentrations of 5 and 10mg/L there were significant differences between 100%MHB, and the other three media tested. **Conclusions:** Bacterial killing by NOSO502 is influenced by media type being less in 100%MHB than in 50%MHB, RPMI or DMEM. Use of 50%MHB maybe optimal for use in *in vitro* pharmacodynamic studies which involve endpoints depended on bacterial killing. This work was funded by IMI Call 16 GNA-NOW programme Grant Agreement Number 853979

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