

Control Number: 2023-A-53-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Pharmacodynamic Evaluation of MK-3402 in Murine Thigh and Lung Infection Models against *Pseudomonas aeruginosa* strains with Metallo- β -lactamases**

Author: **A. Lepak¹, K. Young², D. Li³, D. Andes⁴**; ¹Univ. of Wisconsin Sch. of Med. and Publ. Hlth., Madison, WI, ²West Point, PA, ³Kenilworth, NJ, ⁴Verona, WI

Block:

Background: MK-3402 is a novel metallo- β -lactamase (MBL) inhibitor. We evaluated the pharmacodynamic activity of MK-3402 in combination with imipenem/relebactam against MBL-producing *Pseudomonas aeruginosa* (PsA) strains.

Methods: Checkerboard MIC testing was performed for 6 strains expressing different MBLs (VIM or IMP). MK-3402 threshold concentration with observable *in vitro* improvement in imipenem MIC was used in analysis. Plasma and ELF pharmacokinetics were performed in mice. The neutropenic murine thigh and pneumonia model was utilized. Relebactam was dosed at human simulated 24h AUC. Dose-fractionation (DF) of MK-3402 was performed using fixed imipenem free-drug exposures of 10%, 20% and 40% T>MIC (the stasis, 1- and 2-log kill targets) to determine the MK-3402 PK/PD driver. Efficacy studies were performed against all strains, imipenem was fixed at 10%, 20%, and 40% T>MIC with MK-3402 dose range 0-25 mg/kg/3h. Non-linear regression (Hill equation) was used to analyze the data.

Results: Imipenem MIC was >32 mg/L for all strains and decreased to 1-8 mg/L with MK-3402 and relebactam. MK-3402 threshold concentration occurred at 0.13-0.5 mg/L. MK-3402 T>threshold was the optimal PK/PD driver of efficacy (R²=0.77-0.81). Imipenem/relebactam/MK-3402 achieved cidal endpoints against all strains in the thigh model. Efficacy was dependent on imipenem T>MIC and MK-3402 T>threshold. For example, at traditional carbapenem T>MIC targets for stasis, 1- and 2-log kill, the MK-3402 target T>threshold was similar (~30%) for each endpoint (**BOLDED** in Table). In the lung model, comparable drug exposures led to net stasis, but increased exposures were necessary for cidal endpoints.

Abstract Body:

Model	Imipenem plasma free drug T>MIC (%) exposure	Mean MK-3402 plasma free drug T>threshold (%) for Stasis	Mean MK-3402 plasma free drug T>threshold (%) for 1-log kill	Mean MK-3402 plasma free drug T>threshold (%) for 2-log kill
Thigh	40	7.8	14.1	26.3
	20	9.6	28.7	39.9
	10	36.3	51.4	61.3
Lung	40	2.3	28.9	62.3
	20	6.7	59.9	NA
	10	34.5	NA	NA

NA, a point estimate cannot be reliably determined as too few organisms achieved the endpoint.

Conclusions: MK-3402/imipenem/relebactam demonstrated efficacy against MBL-

producing PsA. DF of the inhibitor while maintaining a fixed beta-lactam exposure allowed us to isolate the PK/PD driver for MK-3402, which was $T > \text{threshold}$. Efficacy depended on both imipenem $T > \text{MIC}$ and MK-3402 $T > \text{threshold}$. In the thigh, MK-3402 free drug $T > \text{threshold}$ of $\sim 30\%$ resulted in stasis or cidal endpoints at appropriate imipenem exposures. Cidal endpoints required a 2-fold increase in MK-3402 exposures for pneumonia. These results will be useful for dosing regimen optimization of MK-3402.

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Control Number: 2023-A-64-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **A Randomized, DoubleBlind, Placebo Controlled Multicenter Study to Evaluate the Efficacy and Safety of CAL02 Administered Intravenously in Addition to Standard of Care in Subjects with Severe Community Acquired Bacterial Pneumonia (SCABP)**

Author Block: **V. Curt**¹, A. Kalil², S. Lajaunias³, S. Minassian⁴; ¹Eagle Pharmaceuticals, Woodcliff lakes, NJ, ²Omaha, NE, ³Combioxin, 1066 Epalinges, Switzerland, ⁴Inclin,Inc, San Mateo, CA
A Randomized, Double-Blind, Placebo Controlled Multicenter Study to Evaluate the Efficacy and Safety of CAL02 Administered Intravenously in Addition to Standard of Care (SOC) in Subjects with Severe Community-Acquired Bacterial Pneumonia (SCABP)

A. Kalil¹, S. Azeredo da Silveira², S.L.Minassian³, **V. Curt**⁴ Univ. of Nebraska, NE; ²Combioxin, CH; ³InClin, CA; ⁴Eagle Pharmaceuticals, NJ.

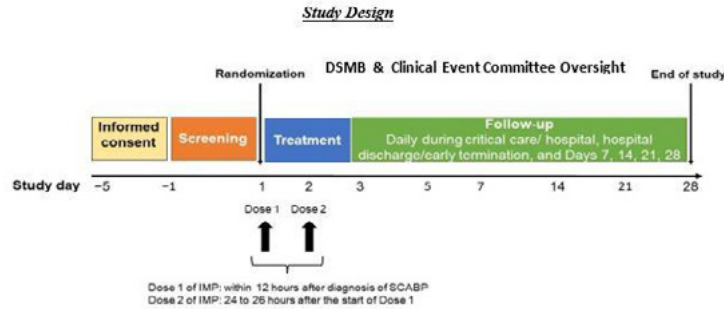
Background : Community-acquired bacterial pneumonia is prevalent worldwide and often fatal. Treatment failures are common and attributed to virulence factors (VFs) and antibiotic resistance. CAL02 is a novel agent designed to neutralize bacterial VFs. The first-in-human study (CAL02-001) showed good safety and efficacy when added to SOC in SCABP patients.

Abstract Body: **Method :** Study **EGL-6535-C-2202**, an adaptive, multicenter, randomized, double-blind, placebo controlled study, will further assess the efficacy, safety, and tolerability of CAL02. Approximately 276 subjects needing critical care for SCABP, across 130 global sites, will be randomized to receive CAL02 or placebo by two IV infusions 24 hrs. apart, along with SOC and will be followed for 28 days. A bracketed dose by weight scheme to account for blood volume differences will be followed.

Primary endpoints will evaluate effect of CAL02 on clinical recovery (resolution of septic shock and/or respiratory failure per protocol and no recurrence or additional severity criteria within 24 hours) and safety/tolerability vs. placebo. Secondary endpoints include time to discharge from critical care/hospital and SOFA score evolution. An independent Data Safety Monitoring Board will monitor safety and efficacy at interim analyses planned at 33% and 50% of enrollment, and a Clinical Event Committee will assess adequacy of SOC.

Conclusion : The results will further the development of CAL02 to address important

unmet clinical needs in SCABP at the individual and public health levels.



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Control Number: 2023-A-70-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Exploring the Effect of Sub-MIC Antibiotic Exposure on Bacterial Hetero-resistance at Single Cell Level**

Author Block: S. Ahmad, A. Samborski, P. Jankowski, S. Vasantham, I. Foik, P. Garstecki; Instytut Chemii Fizycznej PAN, Warsaw, Poland

Abstract Body:

Background: Bacterial cells may present different phenotypes even within monoclonal populations and some confer them selective advantages during stress. On antibiotic exposure, if the individual cells show differential minimum inhibitory concentrations (MICs), they are called hetero-resistant and it is believed to play a major role in selection, evolution and resistance in bacteria. Action of an antibiotic is accompanied by a variety of responses in bacteria, which cumulatively inhibit its growth. However, if the exposure time or concentration is not enough, bacteria might not die but still respond which might generate minor changes in their biomolecules. Such changes may alter the behaviors of these cells on subsequent exposure against the same or a different antibiotic, including hetero-resistance pattern. During antibiotic susceptibility testing (AST) of single cells, a term single cell MIC (scMIC) is introduced which is the population average level of concentration inhibiting the proliferation of individual cells. The probability distribution of scMIC gives an insight into the hetero-resistance of the population.

Methods: Droplet microfluidics is widely used in AST it allows for separation of individual bacterial cells via stochastic confinement while being portable, fast, high-throughput and reagent efficient. Overnight cultures of bacteria were refreshed till OD 0.1-0.2, exposed at sub-MIC or no antibiotic in control, grown till OD 0.1-0.2, encapsulated 1 cell per 1nL droplet with a range of antibiotic concentrations and incubated for 15 hours. Both bright field and fluorescent images were acquired, analyzed, scMIC determined, resistance profile and distribution of scMIC plotted.

Results: Data analysis revealed differences among droplet intensities even within one concentration of antibiotic which directly correspond to how individual cells grew inside one droplet. The scMIC distribution also changed for the cells exposed with antibiotic and the pattern depended on the antibiotic used for exposure as well as re-

exposure.

Conclusions: What doesn't kill you makes you stronger- this line perfectly fits in our study where the bacteria stimulated with low antibiotic concentration showed increased survival. Further studies are needed to establish pattern, if any, based on antibiotic class or mechanism of action. The data from the study can be very crucial for better understanding of the resistance mechanism, thereby helping in its efficient management and new drug development.

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Control Number: 2023-A-74-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Exebacase MIC Determination for Staphylococci Other Than *Staphylococcus aureus* (SoSA)**

Author Block: J. E. Ambler¹, R. Schuch¹, B. L. M. de Jonge¹, J. Difranco-Fisher², L. M. Koeth²; ¹Contrafact Corp., Yonkers, NY, ²Lab. Specialists, Inc., Westlake, OH

Abstract Body:

Background: Exebacase (EXE) is a protein-based antistaphylococcal lysin (peptidoglycan hydrolase) in clinical development. For *S. aureus* EXE MIC determination, a standard broth microdilution (BMD) method using cation-adjusted Mueller-Hinton Broth with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (CAMHB-HSD) is used. When surveillance SoSA isolates were tested previously (n=205) using CAMHB-HSD, a significant proportion, particularly *S. epidermidis* (47%), grew poorly. *S. epidermidis*, a major pathogen in prosthetic joint infection, is known to exhibit serum sensitivity. To overcome SoSA growth limitations in CAMHB-HSD, EXE MIC panels were incubated in 5% CO₂/20-24 h. This study assessed the use of CAMHB-HSD + 5% CO₂/20-24 h for EXE MIC determination against 49 SoSA isolates, of which 30 were selected for method verification testing.

Methods: Exebacase MICs against SoSA (n=49) isolates including *S. epidermidis*, *S. capitis*, *S. caprae*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, and *S. saprophyticus* were determined in triplicate over 3 days using CAMHB-HSD + 5% CO₂/20-24 h. MIC results were read according to 2 different endpoints (Read 1 = according to CLSI M100 *S. aureus* guidance; Read 2 = 100% inhibition). Oxacillin, rifampin and vancomycin MICs were determined using the CLSI reference BMD method. Quality control (QC) strain *S. aureus* ATCC 29213 was tested on each day and incubated in 5% CO₂/20-24 h.

Results: 5% CO₂/20-24 h incubation greatly improved the growth of SoSA that grew poorly in CAMHB-HSD in ambient air/16-20 h. Read 2 end points were easier to read vs Read 1 ($\leq 1 \log_2$ different). Read 2 MIC_{50/90} were 2/8 $\mu\text{g}/\text{mL}$ for all SoSA, 4/8 $\mu\text{g}/\text{mL}$ for *S. epidermidis* (n=29); the MIC range was 2 to 8 $\mu\text{g}/\text{mL}$ for *S. caprae* (n=4) and MICs were $\leq 2 \mu\text{g}/\text{mL}$ for the remaining species tested. MIC ranges to oxacillin, rifampicin and vancomycin were ≤ 0.06 to $>4 \mu\text{g}/\text{mL}$, 0.016 to $>32 \mu\text{g}/\text{mL}$ and 0.5 to 8 $\mu\text{g}/\text{mL}$, respectively.

Conclusions: Growth of SoSA in CAMHB-HSD + 5% CO₂/20-24 h was adequate for EXE MIC determination. 100% inhibition was easier to read vs CLSI guidance for *S. aureus* end points. EXE MIC_{50/90} were 2/8 $\mu\text{g}/\text{mL}$ for all SoSA tested; intra-laboratory MIC reproducibility was acceptable. *S. aureus* QC results generated in 5%

CO₂/20-24 h were within CLSI range. Further testing of SoSA using 5% CO₂/20-24 h is warranted.

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Control Number: 2023-A-76-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Method Verification of Exebacase MIC Method for Staphylococci other than *S. aureus* (SoSA)**

Author Block: **J. E. Ambler¹, J. M. Difranco-Fisher², L. M. Koeth²; ¹Contrafect Corp., Yonkers, NY, ²Lab. Specialists, Inc., Westlake, OH**

Background: Exebacase (EXE) is an antistaphylococcal lysin (peptidoglycan hydrolase). The standard EXE broth microdilution (BMD) method for *Staphylococcus aureus* MIC determination uses cation-adjusted Mueller-Hinton broth with horse serum (25% v/v) and 0.5 mM DL-dithiothreitol (CAMHB-HSD). Prior testing of SoSA showed that 5% CO₂/20-24 h incubation of MIC panels is needed for adequate growth for MIC determination. A 3-site method verification (MV) study was done to assess whether the modification (+ 5% CO₂/20-24 h incubation) to the standard EXE BMD method impacts test performance by testing verification isolates and quality control (QC) strain *S. aureus* ATCC 29213.

Methods: EXE MV isolates comprising 30 SoSA (20 *S. epidermidis*, 2 *S. capitis*, 2 *S. caprae*, 2 *S. haemolyticus*, 2 *S. hominis* and 2 *S. lugdunensis*) were tested in triplicate. A single batch of frozen EXE MIC panels were prepared using CAMHB-HSD with 2 different horse serum (HS) lots. Replicate MIC testing was conducted over 3 d using the modified EXE BMD method (5% CO₂/20-24 h). MICs were read according to 2 different end points (Read 1: CLSI M100 *S. aureus* guidance; Read 2: 100% inhibition). Replicate QC tests were done daily, and incubated + 5% CO₂/20-24 h and + ambient air/16-20 h.

Abstract Body:

Results: A total of 180 MICs/site were generated; Read 2 modal MICs are presented by site and by HS lot (Table). 96.7-100% of modal MICs at each site were within ±1 dilution of the all-site mode. All *S. aureus* ATCC 29213 MICs were within the CLSI QC range (0.25-2 µg/mL).

Conclusions: Good intra- and inter-laboratory reproducibility was observed for all SoSA MV isolates tested. Together with QC MICs, these data demonstrate that the performance characteristics of the modified method are in line with those previously established with the standard EXE BMD method. Future provision of MV isolates to laboratories will allow the generation of data for comparison with this study data. Such analyses allow validation of MIC panels prepared by laboratories using different HS lots prior to testing clinical isolates.

SoSA* modal EXE MIC distributions by test site and horse serum lot (Read 2 end point, 100% growth inhibition)

Horse serum lot	Site #	EXE MIC (µg/mL)					
		0.5	1	2	4	8	16

1	1	1	3	11	7	4	4	
	2	1	3	8	10	3	5	
	3	1	3	6	9	5	6	
2	1	1	4	10	7	5	3	
	2	3	3	7	10	4	3	
	3	2	2	6	12	5	3	
			No. of occurrences at EXE MIC (µg/mL)					
			0.5	1	2	4	8	16
<i>S. aureus</i> ATCC 29213				30	6			
*30 SoSA = 20 <i>S. epidermidis</i> , 2 <i>S. caprae</i> , 2 <i>S. captis</i> , 2 <i>S. haemolyticus</i> 2 <i>S. hominis</i> , 2 <i>S. lugdunensis</i>								

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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

Abstract Body:

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 (≥ 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.

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
<i>P.aeruginosa</i> Total Isolates (N)	49,542	10.12	5,875	18.7	55,417	10.64
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
Enterobacteriales (ENT) Isolates (N)	310,946	63.55	28,511	90.74	339,457	65.19
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
ENT Isolates Tested for ESBL (N)	270,622	55.31	24,353	77.51	294,975	56.65
ESBL + cases (n)	13.4% (36,293)	7.42	15.0% (3,647)*	11.61*	13.5% (39,940)	7.67
Enterococcus spp. Isolates (N)	66,207	13.49	7,268	23.13	73,295	14.08
Vancomycin resistant enterococcus (VRE)	14.6% (9,633)	1.97	17.0% (1,237)*	3.94*	14.8% (10,870)	2.09
<i>S.aureus</i> Isolates (N)	103,015	21.05	8,154	25.95	111,169	21.35
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
Fungal/Yeast Isolates (N)	41,755	8.53	5,864	18.7*	47,619	9.14
<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-85-ASM-ESC

Session Title:

Poster Presentations Session I

Publishing Title:

Oral Pharmacokinetics and Efficacy of BWC0977, a Novel Bacterial Topoisomerase Inhibitor

Author Block:

H. K. Kotakonda, S. Reddy, S. Hameed, V. Balasubramanian; Bugworks Res. Inc., Bangalore, India

Abstract Body:

Background: BWC0977, a broad-spectrum antibiotic, is currently undergoing clinical development as an intravenous drug candidate for critical care infections. The oral route of administration would be beneficial as a step-down following IV and as treatment for community and biothreat infections. BWC0977 is a BCS Class III drug with high P-gp efflux and pH-dependent solubility. Using a lipid-based oil in water dispersion formulations (Self-emulsifying drug delivery systems, SEDDS) with inclusion of permeation enhancers improved oral bioavailability. **Methods:** Thermodynamic equilibrium 24hr solubility at 37°C was measured in water, various pH aqueous buffers, and biorelevant media. The human intestinal permeability was measured bidirectionally using CaCO-2 monolayers. Oral pharmacokinetics were determined in mice, rats, and dogs from 30mg/kg to 300mg/kg following a single dose administration. Aqueous suspensions of Tween80+HPMC and Tween80+CMC were evaluated along with an enabling formulation of Gelucire44/14 +Solutol-HS15+ Vit-E Tocopheryl polyethylene glycol succinate (TPGS), and Propylene glycol (for mice and

rats) in water. Quantification of all samples was done by LC/MS/MS. Oral efficacy was assessed in an mice thigh infection model challenged with *P. aeruginosa*, *A. baumannii*, *E. coli*, *K. pneumoniae*, *E. cloacae* & *S. aureus*. **Results:** In mice, rats and dogs, the SEDDS formulation achieved higher oral exposures compared to the generic suspension formulation (Tween80 & HPMC in water). Dose proportional increase in exposures was observed in all the species. In the fed state, a positive food effect was observed. The SEDDS formulation resulted in the oral bioavailability as follows: 82% in mouse, 30% in rats and 50% in dogs. **Conclusion:** In the mice thigh infection model, BWC0977 showed dose dependent bactericidal efficacy against multiple strains of Gram negative and Gram positive bacteria. The PK/PD index that best described the in vivo efficacy of BWC0977 was fAUC₂₄/MIC across the strains.

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Control Number: 2023-A-88-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **A Novel Fosfomycin-Trimethoprim-Sulfamethoxazole Combination Exhibits Marked In Vitro Antibacterial Synergy Against Bacteria Causative of Urinary Tract Infections**

Author Block: S. Gardlik¹, T. Hartsell², L. Williams³, R. Doyle⁴, M. Villegas⁵, S. Volla⁶, J. Pace⁷; ¹FleurirABX, Raleigh, NC, ²Sentara Hlth.care, Elizabeth City, NC, ³FleurirABX, Raleigh, NC, ⁴NC State Univ., Raleigh, NC, ⁵Univ. del Bosque, Cali,

Colombia, ⁶FleurirABX, Philadelphia, PA, ⁷FleurirABX, Elizabeth City, NC, NJ
BACKGROUND: Increasing incidence/recurrence of urinary tract infections (UTI), particularly in aging populations, can lead to increased antibacterial resistance, complications requiring hospitalization, and exacerbated cognitive decline. Oral fosfomycin (F) has had low use as a treatment for UTI in the U.S. High empiric use of other antibacterials (ciprofloxacin, trimethoprim-sulfamethoxazole (TS), amoxicillin-clavulanate, cephalosporins, nitrofurantoin, ceftriaxone) can select for pathogens with altered targets, decreased transport or producing extended-spectrum beta-lactamases. To address these challenges, we evaluated in vitro activity of F and TS in combination against a selection of causative pathogens. **METHODS:** We utilized the synergy-checkerboard variant of the CLSI agar minimal inhibitory concentration assay with Mueller-Hinton Agar (glucose-6-phosphate supplemented) and evaluated fosfomycin-trimethoprim-sulfamethoxazole (FTS) in vitro against UTI pathogens (*Escherichia coli*, *Proteus mirabilis*, *Staphylococcus saprophyticus*, and *Enterococcus faecalis*). We compared susceptibility to component antibiotics, F and TS, and to ciprofloxacin, nitrofurantoin, 3rd-gen cephalosporins, beta-lactam-beta-lactamase inhibitor combinations, and carbapenems by agar method or broth microdilution with supplemented cation-adjusted Mueller-Hinton Broth. **RESULTS:** FTS MICs (F in combination and TS in combination, respectively) ranged from 0.5 - 32 mg/L, and 0.015/0.3 - 2/38 mg/L against *Escherichia coli*, 0.25 - 16 mg/L and 0.0075/0.15 - 0.5/9.5 mg/L against *Proteus mirabilis*, 8 - 16 mg/L and 0.06/1.19 - 0.125/2.38 mg/L against *Staphylococcus saprophyticus*, and 0.125 - 8 mg/L and 0.5/9.5 - 2/38 mg/L against *Enterococcus faecalis*. Synergy (Fractional Inhibitory Concentration ≤ 0.5) between F and TS in combination was observed against all *E. coli* (N=17), *P. mirabilis* (N=4), *S. saprophyticus* (N=3), and *E.*

Abstract Body:

faecalis isolates (N=4). Remarkably, synergy between F and TS occurred regardless of resistance to F or TS, or to any other antibacterial agent/class tested. **CONCLUSION:** The unique synergistic antibacterial combination FTS may be an important alternative treatment for UTIs, and merits clinical evaluation.

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Control Number: 2023-A-94-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Recombinant Phage-encoded Lysin Exhibits Antibacterial Activity Against Drug-resistant Uropathogenic Escherichia coli**

Author Block: S. Dhar¹, S. Nain¹, U. Gaharwar¹, B. Chavan¹, R. Das¹, S. Hanif¹, U. Bajpai², D. Sikriwal¹, A. Choudhary¹, S. Syeda¹, S. Shah¹, **S. Ahmed**¹; ¹Techinvention Lifecare Private Limited, Mumbai, India, ²Acharya Narendra Dev Coll., University of Delhi, New Delhi, India

Abstract Body: **Background:** The rampant misuse of antibiotics has led to the emergence of multi-drug resistance in uropathogenic *Escherichia coli* (UPEC), which significantly impacts clinical outcomes in urinary tract infection (UTI) patients. Hence, we are exploring phage-encoded lysins as suitable alternatives. Our study involved an *in-silico* strategy for the discovery and characterization of lysin sequences (seq) targeting *E. coli* cell wall and evaluating the bactericidal activity of these recombinant lysins using *in-vitro* assays. **Methods:** Novel lysin sequences were searched by BLAST homology and by screening *E. coli* prophages in the database (using PHASTER). Lysozyme-like domain was observed in 9 out of 16 lysins. Their characterization depicted modular or globular structure. Based on the physicochemical properties, 7 out of 16 lysins were selected for cloning, expression, and purification as recombinant proteins for evaluating the bactericidal activity. **Results:** Among the several lysins screened, lysin seq 5 demonstrated highest activity using *in-vitro* assays. Using static biofilm assay, lysin seq 5 (180 µg) showed efficient reduction (>50%) in the biofilm formed by ATCC UPEC 700928 strain. As per turbidity reduction method, lysin seq 5 (50 µg) showed 37% drop in OD_{600nm} on UPEC 700928 strain after 3 hours of incubation at 37°C. Using pH experiments, lysin seq 5 demonstrated lytic activity (51%) against UPEC 700928 strain and was stable at pH 6. However, the highest activity was observed at pH 9 (59%). Using spot on lawn assay, lysin seq 5 (20 µg) exhibited lytic activity (zone of inhibition) on five drug-resistant clinical UTI isolates, which were pretreated with an outer membrane permeabilizer (OMP), viz., EDTA (0.3 mM). **Conclusion:** Lysin seq 5 exhibited anti-biofilm activity against UPEC 700928 strain as well as lytic activity against drug-resistant clinical UTI isolates. Apart from showing activity at pH 9 against UPEC 700928 strain, lysin seq 5 was stable and demonstrated lytic activity at pH 6 as well. Screening of additional drug-resistant clinical isolates from UTI patients is underway.

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Control Number: 2023-A-95-ASM-ESC

Session Title: Poster Presentations Session I

Publishing Title: In-vitro Antibacterial Activity of a Fosfomycin-Trimethoprim-Sulfamethoxazole Combination Against a Bacterial Collection of Multidrug-Resistant Clinical Isolates

Author Block: T. L. Hartsell¹, S. Adams², L. Williams³, M. V. Villegas⁴, S. Gardlik², S. Volla⁵, J. L. Pace³; ¹Sentara Hlth.care, Elizabeth City, NC, ²FleurirABX, LLC, Raleigh, NC, ³FleurirABX, LLC, Elizabeth City, NC, ⁴CIDEIM, Cali, Colombia, ⁵FleurirABX, LLC, Philadelphia, PA

Abstract Body: **Background:** Treatment of life-threatening infections in the critically ill patient comes with many challenges. Successful antimicrobial therapy is affected by complex pathophysiology impacting drug dosing, tolerable side effect profile, and development of complications. Infections resistant to multiple antibacterial classes are increasing; identification of synergistic antibiotic combinations may yield important new treatment options. The *in-vitro* antibacterial activity of a unique combination of fosfomycin (F) and trimethoprim/sulfamethoxazole (TS) was evaluated against a collection of multidrug-resistant clinical isolates representative of those found in severe pneumonia, complicated intra-abdominal, urinary tract, and skin/soft tissue infections. **Methods:** The synergy-checkerboard variant of CLSI agar minimal inhibitory concentration assay with Mueller-Hinton Agar (glucose-6-phosphate supplemented) was utilized to evaluate fosfomycin + trimethoprim-sulfamethoxazole (FTS) *in-vitro* against a collection of pathogens causing diverse infections: *E. coli* (N=22), *K. pneumoniae* (N=13), *P. aeruginosa* (N=19), *A. baumannii* (N=19), *S. maltophilia* (N=5), *B. cepacia* (N=10), *S. aureus* (N=18), *enterococci* (N=17), and streptococci (N=16). We compared susceptibility to components F and TS alone, or species-appropriate comparators; EUCAST susceptibility breakpoint for parenteral F (< 32 mg/ml) and CLSI/EUCAST susceptibility breakpoint for TS (< 2/38 mg/ml) were used. Fractional inhibitory concentration (FIC) as an indicator of synergy was calculated for FTS combination (Synergy, ≤0.5).

Results: FTS combination demonstrates striking antibacterial activity; synergy was observed against all isolates (FIC) regardless of acquired or intrinsic resistance. FTS MICs ranged from < 0.5 - 64 mg/L (F in combination) and 0.0078/0.015 - 16/304 mg/L (TS in combination) in contrast to F alone (1- > 256 mg/L) or TS alone (0.125/2.38 - > 32/> 608 mg/L). All but 9 of 139 isolates would be considered fully susceptible to FTS (MIC < 32/2/38 mg/L). Susceptibility to FTS was unrelated to resistance to any comparator antibiotics. **Conclusions:** FTS may be an important alternative therapy for treatment of complicated bacterial infections where spectrum and multidrug resistance are challenges.

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Control Number: 2023-A-105-ASM-ESC

Session Title: Poster Presentations Session I

Publishing Title: A Genome Wide Screen Defines Macrophage Genes Modulating Intracellular Control of Mycobacterium abscessus Following Antibiotic Treatment

**Author
Block:**

H. Gilliland¹, A. Olive²; ¹Michigan State Univ., East Lansing, MI, ²East Lansing, MI

**Abstract
Body:**

BackgroundAntibiotics disrupt essential structures or processes in bacteria. For intracellular pathogens like *Mycobacterium abscessus* (Mab), a leading cause of respiratory infection that is highly antibiotic resistant, the mechanisms driving antibiotic efficacy are more complex. Recent research suggests the host cell can play a key role in localizing antibiotics to pathogen-containing vacuoles. Additionally, some antibiotics such as Bedaquiline and Linezolid, are known to directly activate the host to contribute to antibiotic-mediated bacterial killing. Unfortunately, a global understanding of host mechanisms that contribute to antibiotic killing of intracellular pathogens remains unclear, limiting our ability to leverage host pathways to improve bacterial control. We hypothesize that globally defining host genes contributing to host-pathogen-antibiotic interactions using functional genetic approaches will identify important host-directed targets that can be leveraged to improve antibiotic-mediated control of intracellular pathogens. **Methods**To test this hypothesis, we developed a host-pathogen-antibiotic screening platform that couples fluorescent Mab reporters with a genome-wide loss-of-function library in immortalized bone marrow-derived macrophages. Using this platform, we identified host genes that when lost, result in less effective intracellular killing of Mab by either Bedaquiline or Linezolid over 3 days of infection. **Results**Our screens found key roles for host trafficking pathways and host metabolism in the control of intracellular Mab by Bedaquiline and Linezolid, respectively. Thus, preliminary analysis suggests a model where Bedaquiline and Linezolid rely on unique macrophage mechanisms to effectively target Mab during infection. **Conclusions**Follow-up studies using targeted knockout macrophages and chemical inhibitors are defining how these unique macrophage pathways alter Mab survival during Bedaquiline or Linezolid treatment. Furthermore, we are focused on expanding this established pipeline to include other antibiotics and intracellular pathogens to globally identify new contributors of macrophage biology critical to antibiotic-mediated control of intracellular pathogens.

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**Control
Number:**

2023-A-107-ASM-ESC

**Session
Title:**

Poster Presentations Session I

**Publishing
Title:**

LasB Inhibitor Characterization by Novel Lung Homogenate-Based Assays

**Author
Block:**

A. Klein¹, C. Schütz¹, A. Alhayek¹, A. Kany², S. Adam¹, J. Hauptenthal¹, A. Hirsch¹; ¹Helmholtz Inst. for Pharmaceutical Res., Saarbruecken, Germany, ²Helmholtz Inst. for Pharmaceutical Res., Saarbrücken, Germany

**Abstract
Body:**

Background: Addressing the challenge of antimicrobial resistance, new anti-infective agents with advanced modes of action are crucial. Targeting bacterial virulence, specifically the elastase LasB in *Pseudomonas aeruginosa*, shows promise. We investigated a new class of LasB inhibitors in an *ex vivo* lung homogenate assay that reflects the complex *in vivo* interactions involving small molecules, proteins, and cell fragments. Desmosine quantification determined compound inhibitory effects on LasB activity, contributing to the development of innovative anti-infective agents combating

antibiotic-resistant infections. **Methods:** Lung homogenate assays utilized pig lung-derived suspension containing insoluble mature elastin. The small particle size ensured reproducibility and ease of handling. Elastolytic activity was assessed using LC-MS/MS to measure levels of desmosine, a specific elastin marker. The assay was adapted for purified LasB and bacterial cultures, involving bacterial growth and elastase expression within the homogenate. **Results:** All tested compounds showed favorable, dose-dependent activity, with several inhibitors showing activity in the low μM to high nM range. Importantly, compound activity is comparable to previously performed FRET assays. **Conclusions:** With the presented LasB inhibition assay in lung homogenate we have developed a novel tool for the characterization of advanced LasB inhibitors. This ex vivo matrix enables activity assessment in a matrix closer to the scenario of an in vivo infection.

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Control Number: 2023-A-109-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Murine pharmacodynamics of oral avibactam pro-drug in combination with ceftibuten**

Author Block: A. Johnson¹, L. McEntee², **N. Farrington**², I. Horner¹, A. Stevenson¹, J. Unsworth², A. Jimenez-Valverde², R. Kolamunnage-Dona², S. Ripp³, G. Stone⁴, W. Hope¹, S. Das¹; ¹Liverpool, United Kingdom, ²Univ. of Liverpool, Liverpool, United Kingdom, ³Groton, CT, ⁴Franklin, MA

Abstract Body: **Background:** A novel oral pro-drug of avibactam is being developed in combination with ceftibuten. This combination has the potential to be a useful carbapenem-sparing oral agent. Pre-clinical PK-PD studies are needed to identify the appropriate dynamically linked index and magnitude that best links drug exposure with antibacterial efficacy for multidrug resistant Enterobacteriaceae. **Methods:** A murine neutropenic thigh infection model was used to characterise PK-PD of the combination across several clinical ESBL-producing Enterobacteriaceae strains. Subcutaneous doses of ceftibuten were selected to have minimal activity alone but sufficient to show effect in combination. Dose fractionation used a fixed backbone of ceftibuten 25mg/kg q6h and an oral avibactam pro-drug dose of 400 mg/kg was fractionated q24h, q12h or q6h with endpoints at 6, 14 and 26 hours post-infection against *K. pneumoniae* 1466540. For PK both ceftibuten and avibactam pro-drug were administered q6h with plasma samples obtained at 0.25, 0.5, 1, 2, 3, 4, and 6 hours post-dose in the 1st and 4th dosing intervals. To identify magnitude, dose ranging studies were conducted with a fixed backbone of either 25 or 50mg/kg q6h ceftibuten with avibactam pro-drug ranging from 50-750 mg/kg q6h. **Results:** Linear mixed effect models were used to compare the dosing regimens. Significant differences in log₁₀CFU/g change over time ($p < 0.05$) between ceftibuten alone and ceftibuten/avibactam combinations were observed, with no significant differences between each fractionated dose regimen of ceftibuten/avibactam, demonstrating concentration-dependence of avibactam. Stasis was achieved across 7 isolates; MDR *K. pneumoniae*, *E. coli* and *E. cloacae*. Ceftibuten and avibactam showed linear pharmacokinetics in mice. **Conclusions:** Statistical analysis suggested AUC is the relevant dynamically linked PK-PD index used to describe the

pharmacodynamics of oral avibactam pro-drug. PK-PD modelling demonstrated stasis could be achieved with different combinations of $fT > MIC$ targets for ceftibuten and $fAUC$ targets for avibactam.

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Control Number: 2023-A-112-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Repurposing of Drugs to Fight Persistent Infections - Antibacterial Compounds Against Non-growing Bacteria**

Author Block: N. Kaldalu¹, N. Bērziņš¹, S. Berglund Fick², V. Hauryliuk³, T. Tenson¹; ¹Univ. of Tartu, Tartu, Estonia, ²Umeå Univ., Umeå, Sweden, ³Lund Univ., Lund, Sweden

Background: During chronic and recurrent infections, a large fraction of the pathogenic bacteria is non-growing or grows very slowly and escapes antibacterial therapy. For treatment of chronic infections, the drugs that target non-growing bacteria are needed.

Methods: We screened a set of 6480 registered drugs and drug candidates (Prestwick Library and Specs Drug Repurposing Set) for the compounds that are active against non-growing uropathogenic *Escherichia coli* (UPEC). Screening was based on treatment of the stationary phase culture and monitoring delay of outgrowth after dilution of the bacteria into fresh growth medium (Hazan R, et al. BMC Microbiol. 2012; 12:259).

Primary screen was performed in diluted CA-MHB medium (pH7.4) and low phosphate, low magnesium medium (pH5.5) that mimics conditions in the vacuole.

Abstract Body:

Results: 38 compounds of different classes (19 fluoroquinolones, 7 anti-cancer drugs, 5 macrolides, 2 antiseptics, 4 rifamycins, 1 pleuromutilin) delayed regrowth and were tested against the non-growing UPEC for their bactericidal activity at different concentrations. The same set of compounds was tested against non-growing *Pseudomonas aeruginosa* and *Staphylococcus aureus* for delay of regrowth and in a CFU-counting assay. We found several drugs which were strongly bactericidal against the non-proliferating bacteria. Several fluoroquinolones (Clinafloxacin, Sitafoxacin), Mitomycin C, and Solithromycin killed more than four orders of magnitude of non-growing *P. aeruginosa* at 2.5 μM concentration. Alexidine hydrochloride and Mitomycin C killed more than 4 orders of magnitude of non-growing *S. aureus* at 10 μM concentration.

Conclusions: Several approved antibiotics and drug candidates express activity against non-growing bacterial pathogens *in vitro* and could be tested in comparison with current treatments for their efficacy against persistent infections.

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Control Number: 2023-A-129-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: Epidemic *Clostridioides difficile* Isolates are Significantly More Lethal and Persist at Higher Rates than Non-Epidemic Isolates in Hamsters Following Vancomycin Treatment

Author: M. Pulse¹, J. Vitucci², W. Weiss¹, J. Simecka¹;

Block: ¹UNTHSC, fort worth, TX, ²Rockville, MD

Abstract Body: **Background:** Previous reports have suggested that epidemic *Clostridioides difficile* ribotypes are hypervirulent and that their characteristics can dramatically impact clinical disease. Some evidence has shown that epidemic ribotypes have a higher rate of sporulation than non-epidemic isolates, which is thought play a role in the persistence of *C. difficile* in recurrent infections. Support for this association has largely been investigated *in vitro*, but not *in vivo*. Therefore, studies were conducted in a hamster model of CDI to evaluate the virulence of 13 epidemic and non-epidemic isolates as well as their abilities to persist following vancomycin treatment in the model. **Methods:** Median lethal dose (LD₅₀) studies involved orally infecting male Golden Syrian hamsters with 6 epidemic or 7 non-epidemic isolates in a titer range of 800 to 30,000 spores per isolate, and persistent infection studies involved orally infecting hamsters with ~4.7 log₁₀ spores of 2 vancomycin-sensitive epidemic or non-epidemic isolates. Clindamycin (10 mg/kg) was SC administered 24 hours after infection, and for persistent infection studies, vancomycin (20 mg/kg) was orally administered once daily for 5 days starting 18 hours after dosing clindamycin. Feces were daily collected from sterile cages to determine CFU/spore and toxin titers, and survival census was recorded up to 11 days post-infection. **Results:** Mean LD₅₀ values in the hamster CDI model were 3.56 and 3.97 log₁₀ spores for epidemic and non-epidemic isolates, respectively, and these values were determined to be significantly different by the extra sum-of-squares F test ($p \leq 0.05$). Mean toxin titers associated with feces collected from epidemic infected hamsters had up to 2-4 times more Toxin A and B within 4 days of infection as compared to hamsters infected with non-epidemic isolates. Epidemic isolates also had increased persistence in hamsters with 3 times more spores remaining in their feces at the end of vancomycin treatment as compared to non-epidemic infected hamsters. **Conclusions:** These results suggest that epidemic *C. difficile* isolates can persist in the gastrointestinal tract at a higher rate than non-epidemic isolates following treatment, which can lead to higher rates of severe recurrent infections in at risk patients.

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Control Number: 2023-A-130-ASM-ESC

Session Title: Poster Presentations Session I

Publishing Title: Development of Rabbit Models of Ventilator-Associated Carbapenem-Resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Pneumonia

Author Block: V. Petraitis¹, R. Petraitiene¹, P. Kavaliauskas¹, E. Naing¹, A. Garcia¹, V. Zigmantaite², R. Grigaleviciute², A. Kucinskas², R. Stakauskas², T. Walsh³; ¹Weill Cornell Med. of Cornell Univ, New York, NY, ²Lithuanian Univ. of Hlth.Sci., Kaunas, Lithuania, ³Ctr. for Innovative Therapeutics and Diagnostics, Richmond, VA

Abstract Body: **Background:** Development of new antimicrobial agents to effectively treat ventilator-associated bacterial pneumonia (VABP) caused by carbapenem-resistant (CR) Gram-

negative bacteria is paramount. However, current conventional animal model systems have been inadequate to address this pressing need. We therefore developed two rabbit models of VABP caused by CR *Pseudomonas aeruginosa* and CR *Acinetobacter baumannii* and subsequently assessed the efficacy of ceftazidime/avibactam (CAZ-AVB) in these novel systems.

Methods: A novel endotracheal tracheostomy system was developed for intubation in persistently neutropenic NZW rabbits. Each anesthetized rabbit received a predetermined endotracheal inoculum of 1.0×10^8 CFU in a volume of 350-450 μ L. The early-phase intubated model (0-24hrs) received mechanical ventilation and the late-phase intubated model (72-96hrs) was ambulatory. Efficacy of CAZ-AVB was studied in late-phase rabbits with a dosage of 120/30mg/kg IV Q8h. Treatment was initiated 12hrs after inoculation and continued for 4 days. Outcome parameters are delineated in Table 1.

Results: Table 1. Outcome Parameters of the Rabbit Models of CR *Pseudomonas aeruginosa* and CR *Acinetobacter baumannii*

Outcome Variables	Study Cohort			
	<i>Pseudomonas aeruginosa</i>		<i>Acinetobacter baumannii</i>	
	Control(n=4)	CAZ-AVB(n=4)	Control(n=4)	CAZ-AVB(n=4)
Survival	2(50)	4(100)	2(50)	4(100)
Mean residual tissue bacterial burden(log CFU/g \pm SEM)	9.73 \pm 0.16	0.62 \pm 0.05*	7.85 \pm 0.19	0.39 \pm 0.24*
Mean residual BAL bacterial burden(log CFU/mL \pm SEM)	8.71 \pm 0.07	0.00 \pm 0.00*	7.30 \pm 0.47	0.00 \pm 0.00*
Mean lung weight(g)	29.8 \pm 4.87	23.3 \pm 0.48	25.3 \pm 1.44	22.0 \pm 2.52
Mean pulmonary lesion score	5.75 \pm 0.25	0.50 \pm 0.29¶	4.75 \pm 0.25	0.50 \pm 0.29¶
Quantitative PCR from lung tissue	13.4 \pm 1.10	10.9 \pm 0.13	9.08 \pm 0.09	8.09 \pm 0.59
Quantitative PCR from BAL	9.44 \pm 0.13	8.67 \pm 0.23	8.79 \pm 0.12	7.20 \pm 0.37
Proinflammatory Cytokines				
serum IL-4(PG/ML \pm SEM)	507 \pm 26.9	305 \pm 34.2*	889 \pm 105	428 \pm 75.3*
BAL IL-4(PG/ML \pm SEM)	511 \pm 58.6	458 \pm 40.5	523 \pm 41.1	219 \pm 69.6*
serum IL-6(PG/ML \pm SEM)	651 \pm 247	224 \pm 107	69.3 \pm 12.5	20.3 \pm 3.11*
BAL IL-6(PG/ML \pm SEM)	1216 \pm 273	693 \pm 301	1946 \pm 519	127 \pm 53.7*
BAL TNF- α (PG/ML \pm SEM)	168 \pm 31.9	57.1 \pm 14.1*	161 \pm 44.1	147 \pm 29.1
BAL IL-1 β (PG/ML \pm SEM)	378 \pm 85.3	155 \pm 17.9*	494 \pm 215	161 \pm 18.4
BAL IL-2(PG/ML \pm SEM)	22.5 \pm 6.34	6.26 \pm 1.98*	11.7 \pm 3.26	7.79 \pm 3.30

*, $P \leq 0.05$; ¶, $P \leq 0.001$.

Discussion. These data demonstrate efficacy of CAZ-AVB in both VAPB CR models with differences between treated animals and untreated controls. The most distinctive differences were observed in survival, residual tissue bacterial burden, lung weights, pulmonary lesion scores, and the following cytokines: serum IL-6, as well as BAL IL-6, TNF- α , IL-1 β , and IL-2.

Conclusions: The new rabbit models of VABP produced by CR *Pseudomonas aeruginosa* and CR *Acinetobacter baumannii*, which recapitulate the pathophysiological, microbiological, diagnostic imaging, and histological patterns of human disease, are effective tools for studying antimicrobial agents developed for treatment of these frequently fatal infections in patients.

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Control Number: 2023-A-133-ASM-ESC

Session Title: **Poster Presentations Session I**

Publishing Title: **Exploring Potential MCR-1 Inhibitors: Restoring *In Vitro* Polymyxin Activity in MCR-1-Producing *Enterobacteriales***

P. Kavaliauskas¹, W. Castillo², R. Petraitiene³, F. Opazo⁴, V. Mickevicius⁵, B. Grybaite⁵, V. Petraitis³;

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¹Weill Cornell Medicine of Cornell University, New York city, NY, ²Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile, ³Weill Cornell Medicine of Cornell University, New York, NY, ⁴Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile, ⁵Kaunas University of Technology, Kaunas, Lithuania

Abstract Body:

Background: The emergence of plasmid-encoded mobile colistin resistance determinants (MCR-1) in Gram-negative pathogens poses a profound healthcare threat. Therefore, it is critical to develop novel inhibitors that are able to restore the susceptibility to polymyxins in MCR-1 strains with pre-existing resistance to carbapenems. **Methods:** A medium-throughput small molecule library (750 compounds), containing various drug-like heterocyclic compounds was used for the screening. The inhibitory properties of compounds were determined by using spectrophotometric whole-cell assay with *E. coli* Dh5a having pPKMCR1-GFP expressing functional *mcr-1* and GFP. The *in vitro* antimicrobial activity was characterized using CLSI recommendations. The crystal structure of MCR-1 was retrieved from Protein Data Bank (code PDB: 5YLE). Ligands and protein were prepared using AutoDock Tools 1.5.7. We selected a volume of 60 × 60 × 60 grid points, enough to cover each receptor. Finally, the binding site and energies ($\Delta G_{binding}$) of each compound were predicted into protein using Autodock Vina. The graphic analysis of the molecular coupling studies was performed using Visual Molecular Dynamics 1.9 and BIOVIA Discovery Studio Visualizer. **Results:** Six out of 750 compounds were able to restore the *E. coli* pPKMCR1-GFP sensitivity to polymyxin B by inhibiting the growth by 50% at fixed 100 μ M concentration. Identified compounds significantly restored the susceptibility of Mcr-1/carbapenemases co-producing *K. pneumoniae* and *E. coli* to polymyxin B (MIC 1-4 μ g/mL) in comparison to polymyxin B alone (MIC 4-16 μ g/mL) in dose-dependent manner ($p < 0.05$). Compounds **86** and **92** demonstrated

strongest activity by decreasing the MIC of polymyxin B by two folds in Mcr-1/carbapenemases co-producing strains. Docking studies showed that compounds **92** and **86** have good $\Delta G_{binding}$ for MCR-1, with values of -5.7 and -5.0 Kcal/mol, respectively. We also showed that these compounds lie in the same binding cavity sharing a set of amino acids, including Tpo70, Glu148, Cys149, Met177 and Arg187. Additionally, the results showed that **92**-binding regions overlap with the catalytic sites of MCR-1, sharing sets of common contacts with ethanolamine, including Tpo70 and Met177. **Conclusions:** Small molecule library screening identified two promising Mcr-1 inhibitors for hit-to-lead optimization. Further studies are needed to understand better the pharmacological activity, safety and efficacy of novel Mcr-1 inhibitors.

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Control Number: 2023-A-119-ASM-ESC

Session

Title:

Publishing Title: Rational design of membrane active antimicrobials against multi-drug resistant Gram negative bacteria

Author Block: E. Lim, J. Li, C. Yang, J. Tan, Y. Yang, C. Verma; Agency of Sci., Technology and Res. (A*STAR), Singapore, Singapore

Abstract Body:

Background: Antibiotics have revolutionized the healthcare and medical landscape since they were first discovered. However, in past decades, multidrug resistant bacteria have come into the spotlight with the increasing number of untreatable infections. This renders many previously well-relied drugs obsolete and poses a threat to global health. The bacterial membrane is a perfect target for the development of new antibiotics. As the outermost layer of the cell, access cannot be easily prevented. It is also limited in its modifications without sacrificing functions optimized through millennia of evolution. Lastly, it can be targeted from outside the cell, adding an additional layer of protection to the drug from potential inactivation modifications.

Methods: Our study capitalises on this aspect, designing potential molecules that can be used to target the negatively-charged bacterial membrane. We use a pharmacophore model involving five components: a hydrophobic scaffold (HS), two hydrocarbon linkers (HL), and two cationic groups (CG), resulting in bola-amphiphilic molecules with a topology of CG-HL-HS-HL-CG. The dual positively-charged ends and the hydrophobic body enable the molecules to insert into the bacterial membrane, resulting in a transmembrane conformation that perturbs both the head groups and the lipid tails of the membrane. We explore effects of differing the scaffold, cationic groups and linker lengths on the antibacterial properties of the molecule, as well as, the synergistic effects of such compounds with last-line polymyxin antibiotics against resistant gram-negative bacteria.

Results: Thus far, promising results have been yielded. The synthesised compounds successfully displayed antibacterial activities against both gram-positive and gram-negative bacteria. The compounds have also displayed high synergy with last-line polymyxin E (colistin) against a panel of colistin-resistant gram-negative bacteria with mcr-1 mutation, successfully reducing the minimum inhibitory concentration (MIC) of colistin to acceptable levels. Preliminary tests on the mechanism of action of the compounds confirmed their membrane-targeting mechanism.

Conclusions: This project reaffirms the promise of the bacterial cell membrane as a target for new antibiotics. It also validates the versatility and flexibility of the pharmacophore model in the compound design and synthesis process. This could accelerate the development of new antibacterials to tackle the issue of antibiotic resistance.