

**Session: P-71. Treatment of Antimicrobial Resistant Infections**

**Background.** *Aspergillus fumigatus* is the leading cause of invasive aspergillosis (IA), a lethal infection among immunocompromised patients. Guideline-recommended antifungal therapy against IA is a triazole antifungal, with other secondary options including an echinocandin and amphotericin B. Concerns about drug-host toxicity and antifungal resistance have been globally reported, so new, safe, and effective therapeutics are imperative.

**Methods.** *In vitro*, CLSI standards were upheld as we tested APX2041, voriconazole, caspofungin, and amphotericin B against various *A. fumigatus* strains. *In vivo* we assessed toxicity and efficacy of APX2104 in immunocompromised mice respectively. Neutropenia was induced with 150 mg/kg of cyclophosphamide on days -2/+3 and 250 mg/kg of cortisone acetate on days -1/+6. Immunocompromised mice were infected in an inhalation chamber via 12 mL of aerosolized spores of *A. fumigatus* CEA10 at a concentration of  $1 \times 10^9$  spores/mL (Day 0). Treatment started day +1 and ended day +7.

**Results.** *In vitro*, APX2041, the active-form of APX2104, has over a 16-fold lower minimum effective concentration (MEC) when compared to voriconazole, caspofungin, and amphotericin B against various *A. fumigatus* strains, including echinocandin- and azole-resistant strains.

*In vivo*, given preliminary pharmacokinetic data, APX2104 was tested in non-infected immunocompromised mice at 60 mg/kg and 78 mg/kg once per day (QD). Deaths due to toxicity were seen only at a dose of 78 mg/kg, so 60 mg/kg was set as a safe dose for our *in vivo* efficacy studies. In IA-challenged neutropenic mice, treatment with either posaconazole (20 mg/kg BID) or APX2104 (60 mg/kg QD) equally prolonged survival in 14 of 15 (93%) mice 14 days post-infection ( $p = 0.985$ ). Untreatment control yielded a survival of 3 of 15 (20%) 14 days post-infection ( $p < 0.001$ ). Consistent with our survival studies, H&E and GMS histological samples also demonstrated that APX2104 treatment decreased fungal burden within the lungs of neutropenic mice when compared to the untreated group.

**Conclusion.** Future studies will test the efficacy of APX2104 and posaconazole against azole antifungal resistant strains *in vivo*, as our preliminary findings suggest that APX2104 is a plausible solution to cure IA disease and combat antifungal resistance.

**Disclosures.** All Authors: No reported disclosures

**1615. Isolation of Lytic Bacteriophages with Broad Host Range Activity Against *Pseudomonas aeruginosa* Strains Isolated from Respiratory Samples from Cystic Fibrosis Patients Intended for Therapeutic Application**

Jose Alexander, MD<sup>1</sup>; Daniel Navas, MLS(ASCP)<sup>1</sup>; Marly Flowers, n/a<sup>2</sup>; Angela Charles, MLS (ASCP)<sup>2</sup>; Amy Carr, PharmD<sup>1</sup>; <sup>1</sup>AdventHealth Orlando, Orlando, FL; <sup>2</sup>AdventHealth, Orlando, Florida

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**Background.** With the rise of the antimicrobial resistance between different genera and species of bacteria, Phage Therapy is becoming a more realistic and accessible option for patients with limited or no antimicrobial options. Being able to have rapid access to a collection of clinical active phages is key for rapid implementation of phage therapy. The Microbiology Department at AdventHealth Orlando is performing routine screening of environmental and patient samples for isolation of phages against non-fermenting Gram negative bacteria to develop a Phage Bank.

**Methods.** Protocols for phage isolation from environmental sources such as lakes, rivers and sewers and clinical samples were developed. A series of respiratory, throat, stool and urine samples were processed following an internal protocol that includes centrifugation, filtration and enrichment. Clinical samples were centrifuged for 10 minutes, filtered using 0.45µm centrifugation filters, seeded with targeted host bacteria (clinical isolates) and incubated at 35°C for 24 hours. The enriched samples were centrifuged and filtered for a final phage enriched solution. Screening and isolation were performed using the Gracia method over trypticase soybean agar (TSA) for plaque morphology and quantification. Host range screening of other clinical isolates of *P. aeruginosa* was performed using the new isolated and purified phages.

**Results.** 4 lytic phages against clinical strains of *P. aeruginosa* from patient with diagnosis of cystic fibrosis (CF), were isolated and purified from 4 different respiratory samples, including sputum and bronchial alveolar lavage. All phages showed phenotypic characteristics of lytic activity. 1 phage was active against 4 strains of *P. aeruginosa*, 1 phage was active against 2 strains of *P. aeruginosa* and the remaining 2 phages were active only against the initial host target strain.

**Conclusion.** With this study we demonstrated the potential use of clinical samples as source for isolating active bacteriophages against clinically significant bacteria strains. Clinical samples from vulnerable population of patients with chronic infections are part of our routine “phage-hunting” process to stock and grow our Phage Bank project for future clinical use.

**Disclosures.** All Authors: No reported disclosures

**1616. Mechanism of Thrombocytopenia Induced by Oxazolidinone Antibiotics (Linezolid, Tedizolid): Demonstration of Impairment of Megakaryocyte Differentiation From Human Hematopoietic Stem Cells associated with Mitochondrial Toxicity**

Paul M. Tulkens, MD, PhD<sup>1</sup>; Tamara V. Milosevic, PhD<sup>1</sup>; Gaëlle Vertenoel, MD<sup>3</sup>; William Vainchenker, MD, PhD<sup>3</sup>; Stefan N. Constantinescu, MD, PhD<sup>2</sup>; Françoise Van Bambeke, PharmD, PhD<sup>1</sup>; <sup>1</sup>Université catholique de Louvain, Bruxelles, Brussels Hoofdstedelijk Gewest, Belgium; <sup>2</sup>Univesrité de Paris-Sud, Villejuif, Ile-de-France, France

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**Background.** Linezolid causes thrombocytopenia, which limits its use. In cell culture and in tissues from treated patients, linezolid impairs mitochondrial protein synthesis (due to structural similarities and common binding sites between bacterial and mitochondrial ribosomes). Recent studies have shown that mitochondria act as a key relay in the process leading from activation of the thrombopoietin receptor to megakaryocytes differentiation.

**Methods.** Validated ex-vivo human model of hematopoietic stem cells (HSC) differentiation for (i) measuring megakaryocytes, granulocyte-monocytes, and burst-forming unit-erythroids colony formation; (ii) differentiation into megakaryocytes (conversion of CD34+ into CD41+/CD42+ cells; morphology) and proplatelets formation, (iii) mitochondrial toxicity (electron microscopy; cytochrome c-oxidase activity [partly encoded by the mitochondrial genome]).

**Results.** We show that linezolid (and the recently approved tedizolid), both at concentrations corresponding to their human serum concentrations) inhibit the maturation of HSC into fully differentiated megakaryocytes (CD41 and CD42-positive cells) and the formation of proplatelets. Optic and Electron microscopy showed an impairment of the formation of typical megakaryocytes (lack of large polylobulated nuclei and of intracellular demarcation membrane system [required for platelet formation]), together with disappearance of the internal structure of mitochondria. Biochemical studies showed a complete suppression of the activity of cytochrome c-oxidase (a key enzyme of the mitochondrial respiratory chain).

**Conclusion.** Our study provides for the first time insights in the mechanism of thrombocytopenia induced by linezolid and tedizolid, identifying mitochondria as their target and showing that the drugs will impair the differentiation of hematopoietic stem cells into mature platelets-releasing megakaryocytes. It illustrates how mitochondria dysfunction may play a key role in toxicology and diseases, while paving the way for rational approaches for the design and screening of less toxic derivatives for the benefit of future patients.

**Disclosures.** Paul M. Tulkens, MD, PhD, Bayer (Consultant, Advisor or Review Panel member, Speaker's Bureau) Menarini (Speaker's Bureau) Merck (Advisor or Review Panel member, Speaker's Bureau) Trius (now part of Merck) (Advisor or Review Panel member, Research Grant or Support) Françoise Van Bambeke, PharmD, PhD, Bayer (Speaker's Bureau)

**1617. Mecillinam susceptibility against Enterobacteriales isolated from urinary tract infections from US patients in 2018**

Stephen Hawser, PhD<sup>1</sup>; Ian Morrissey<sup>2</sup>; Cedric Charrier, PhD<sup>2</sup>; Cyntia De Piano, PhD<sup>1</sup>; Morton Alexander, PhD<sup>2</sup>; Anne Santerre Henriksen, MS<sup>4</sup>; IHMA, Monthey, Valais, Switzerland; <sup>2</sup>IHMA Europe, Monthey, Valais, Switzerland; <sup>3</sup>Utility Therapeutics, London, England, United Kingdom; <sup>4</sup>Maxel Consulting ApS, London, England, United Kingdom

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**Background.** Mecillinam is a unique amidinopenicillin antibiotic, being the first and the only compound in its class. In contrast to other beta-lactams, it has a unique mechanism of action whereby it exerts its antibacterial activity through binding to penicillin binding protein 2. Pivmecillinam is the oral-prodrug of mecillinam and recommended as a first line therapy in the IDSA guidelines for uncomplicated urinary tract infections (uUTI), despite not yet being available in the USA. To support the clinical development of mecillinam and pivmecillinam in the USA for the treatment of both complicated UTI and uUTI this study investigated the activity of mecillinam against Enterobacteriales isolates from the USA during 2018.

**Methods.** A total of 1,090 isolates from urinary tract infections from patients in the USA were tested. Activity of antibiotics was tested by CLSI methodology and susceptibility interpreted according to CLSI guidelines.

**Results.** Susceptibility and activity of each antibiotic are shown in the Table. Mecillinam MIC<sub>50</sub> and MIC<sub>90</sub> were 0.25 and 4 µg/mL, respectively and 94.5% of isolates were susceptible. Fosfomycin MIC<sub>50</sub> and MIC<sub>90</sub> were 2 and 32 µg/mL, respectively and 95.7% of isolates were susceptible. The other four comparator antibiotics showed MIC<sub>90</sub> values >8 µg/mL and a 70.5 – 79.9% susceptible isolates. The highest MIC<sub>90</sub> against all isolates combined was 64 µg/mL for nitrofurantoin and the highest percentage of resistance was obtained with trimethoprim-sulfamethoxazole with 29.5%. Resistance towards ceftriaxone and ciprofloxacin was 19.6% and 26.1%, respectively.

Table

Drug	Breakpoints (S  R)	Susceptibility			MIC (µg/mL)			
		%S	%I	%R	MIC <sub>50</sub>	MIC <sub>90</sub>	MIN	MAX
MEC	≤8   16   ≥32	94.50	1.5	4.0	0.25	4	0.03	>128
CRO	≤1   2   ≥4	79.9	0.5	19.6	0.03	>8	≤0.015	>8
CIP	≤0.25   0.5   ≥1	71.5	2.5	26.0	0.015	>8	≤0.002	>8
FOS	≤64   128   ≥256	95.7	2.3	2.0	2	32	≤0.06	>256
NIT	≤32   64   ≥128	70.6	19.8	9.5	16	64	≤2	>128
SXT (1:19)	≤2/38   -   ≥4/76	70.5	-	29.5	0.12	>8	≤0.015	>8

MEC, mecillinam; CRO, ceftriaxone; CIP, ciprofloxacin; FOS, Fosfomycin; NIT, nitrofurantoin; SXT (1:19), trimethoprim / sulfamethoxazole (1:19)

**Conclusion.** Overall, mecillinam showed the lowest MIC<sub>90</sub> and a comparable susceptibility profile (94.5 % susceptible and 4.0 % resistant) to fosfomycin (i.e. 95.7% and 2.0% resistant) susceptible isolates). Resistance to ceftriaxone, ciprofloxacin and trimethoprim/sulfamethoxazole around or above 20% is concerning for their clinical usage to treat urinary tract infections. These encouraging susceptibility data warrant