J Antimicrob Chemother
https://doi.org/10.1093/jac/dkaf100

Journal of Antimicrobial Chemotherapy 50 years

Quantitative performance of humanized plasma and epithelial lining fluid exposures of meropenem, cefiderocol and tobramycin against a challenge set of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in a standardized neutropenic murine pneumonia model

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Received 9 December 2024; accepted 10 March 2025

Background: The COMBINE murine neutropenic pneumonia model looks to standardize an important element of preclinical development and provide interlaboratory uniformity. Herein we provide quantitative bacterial density in lung benchmark efficacy data of humanized exposures of meropenem, cefiderocol and tobramycin in plasma and epithelial lining fluid (ELF) against a collection of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Methods: In accordance with the COMBINE protocol, human-simulated regimens (HSRs) based on both plasma and ELF exposures of meropenem, cefiderocol (both as 2 g q8h as 3 h infusions) and tobramycin 7 mg/kg as 30 min infusions were tested against *K. pneumoniae* and *P. aeruginosa* isolates. The 24 h change in cfu/lung for each HSR was calculated. Each isolate was tested in duplicate against both the plasma and ELF HSRs on separate experiment days.

Results: Meropenem HSRs demonstrated >1 $\log_{10}$ kill against all *P. aeruginosa* isolates with MICs of ≤16 mg/L, but only against *K. pneumoniae* isolates with MICs of ≤2 mg/L as isolates with MICs of >2 mg/L generally harboured carbapenemases. Cefiderocol HSRs uniformly achieved >1 $\log_{10}$ kill against both species at MICs of ≤8 mg/L, with net growth and extensive variability in *P. aeruginosa* isolates with MICs of 16 mg/L. All tobramycin-susceptible isolates demonstrated >1 $\log_{10}$ kill, while non-susceptible isolates did not. Differences in cfu/lung magnitude between the plasma and ELF HSRs were most pronounced around the clinical breakpoints.

Conclusions: In the COMBINE pneumonia model, administration of plasma and ELF HSRs of meropenem, cefiderocol and tobramycin demonstrated 24 h cfu/lung within reason of expectation given known PK/PD properties and existing clinical breakpoints.

Introduction

Preclinical pharmacodynamic (PD) infection models play an integral role in the development of novel anti-infective compounds.[1] However, the absence of standardization within these models between laboratories is a complicating factor in clinical translation and benchmarking.[2] Establishing some level of model consistency is paramount to levelling the preclinical playing field and providing a means to better ensure reproducible findings.[3,4] To that end, the Collaboration for prevention and treatment of MDR bacterial infections (COMBINE) project has developed a standardized protocol for the murine neutropenic pneumonia model.[5] Prior efforts have established a diverse phenotypic and genotypic challenge set of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates suitable for use in this standardized model.[6] The isolates have reproducibly demonstrated stable MIC values via broth microdilution (BMD), consistent recovery after inoculation and establishment of sufficient growth over the duration of study.

Traditional preclinical pharmacokinetic/PD (PK/PD) dose ranging and fractionation studies are important in informing clinical dose selection in humans. However, these studies ultimately tend to describe single traditional PK/PD indices (i.e. AUC/MIC,

C_{max}/MIC, or $\%T_{>MIC}$) without appreciating the collinearity or co-dependence between all of them on predicting efficacy. As such, simulating the human profile of compounds allows for the additional clinical translation that antimicrobial efficacy is sustained at clinically relevant exposures.[7] Another important attribute to the clinical translation of preclinical animal infection models is the careful consideration of exposures at the site of infection; in the case of the pneumonia model this is the pulmonary epithelial lining fluid (ELF). It has been well described that interspecies differences in target site penetration, including ELF, commonly exist and therefore considering only plasma concentrations may lead to relative overexposure or underexposure at the infection site in mice.[8] To this end, we endeavoured to develop within the COMBINE murine neutropenic pneumonia model human-simulated regimens (HSRs) separately in both plasma and ELF for five antibiotics representative of different antibiotic classes. With HSRs pharmacokinetically confirmed, they were tested against the *K. pneumoniae* and *P. aeruginosa* challenge set to provide robust quantitative benchmarks using compounds representative of many antibiotic classes for future compound development. The cfu/lung benchmarks for HSRs of tigecycline and levofloxacin have been previously reported.[9] Herein, we provide the cfu/lung benchmarks for HSRs of the remaining compounds: meropenem, cefiderocol and tobramycin. The development and confirmation of the HSRs in the model have been previously described.[10]

Materials and methods

Antimicrobial agents

Commercial vials were acquired as follows: meropenem 1 g (PremierProRx, Lot #0004E21), cefiderocol 1 g (Shionogi, Lot #0021), tobramycin 80 mg/2 mL (Mylan, Lot #7608713). Vials were reconstituted as necessary per manufacturer's instructions and further diluted with normal saline to achieve concentrations required to deliver weight-based dosing to the mice.

Bacterial isolates

K. pneumoniae and *P. aeruginosa* isolates were procured from the isolate repositories at the Center for Anti-Infective Research and Development (CAIRD) (Hartford, CT, USA), the CDC and FDA Antibiotic Resistance Isolate Bank (CDC Bank) (Atlanta, GA, USA), the Paul Ehrlich Institute (PEI) (Berlin, Germany) and the Leibniz Institute (DSMZ) (Brunswick, Germany). Prior to experimentation, each isolate was subcultured twice on Trypticase soy agar with 5% sheep blood (Becton Dickinson and Co., Sparks, MD, USA) and incubated at 37°C for approximately 16 h. All isolates will be made readily available for investigators through the DSMZ.

Laboratory animals and the neutropenic pneumonia model

The COMBINE protocol was adhered to with laboratory-specific methods as follows.[5] Specific pathogen-free CD-1, 6–8 week-old female mice were obtained from Charles River Laboratories, Inc. (Raleigh, NC, USA). All animals were housed as groups of six at controlled room temperature in HEPA-filtered cages (Innovive, San Diego, CA, USA). Animals underwent an acclimatization period of 72 h prior to any study procedures. Food and water was provided *ad libitum* and diurnal cycles (12 h light/12 h dark) were maintained within study rooms.

Neutropenia was achieved through intraperitoneal administrations of cyclophosphamide 150 mg/kg on Day −4 and an additional 100 mg/kg on Day −1. A predictable degree of renal impairment was produced using 5 mg/kg of uranyl nitrate administered intraperitoneally on Day −3. The final inoculum was produced from suspension of bacterial colonies from the overnight culture plate in normal saline. Mice were anaesthetized using inhaled isoflurane, manually restrained upright, and infected with 50 µL of bacterial suspension via the nares to target starting bacterial lung burdens of 10^6 for *P. aeruginosa* and 10^7 for *K. pneumoniae* isolates, as previously described.[6] Each bacterial inoculation suspension was utilized within 30 min of initial preparation.

In vivo efficacy studies

Controls were sacrificed 2 h following inoculation and just prior to antibiotic initiation (0 h controls) and 24 h later. All groups contained six mice except for 24 h controls, which contained 4–6 mice. Meropenem, cefiderocol or tobramycin plasma and pulmonary ELF HSRs were administered over 24 h (or equal volume of normal saline in controls), then animals were euthanized by CO_2 asphyxiation followed by cervical dislocation. Lungs from individual animals were aseptically harvested and homogenized in normal saline. Homogenized tissue was then serially diluted 10-fold and 50 µL was plated for cfu/lung quantification with a lower limit of quantification of 1×10^2 cfu/lung. The change in cfu/lung count over 24 h for each treatment was calculated relative to the initial bacterial burden. Each isolate was tested in duplicate against both the plasma and ELF HSRs on separate experiment days to incorporate interday model variability. The raw data from individual experiments were combined and analysed as a whole. Among isolates that were tested against multiples of the three drugs, each drug was tested separately on independent study days.

Meropenem plasma and ELF HSRs were tested against 10 *K. pneumoniae* and 9 *P. aeruginosa* isolates with meropenem MICs previously determined by BMD ranging from ≤0.063 to >64, and 1 to >64 mg/L, respectively. Cefiderocol plasma and ELF HSRs were assessed against 10 *K. pneumoniae* isolates (cefiderocol MIC range: 0.125 to >32 mg/L) and 10 *P. aeruginosa* isolates (cefiderocol MIC range: 0.063–16 mg/L). Tobramycin HSRs were evaluated against six *K. pneumoniae* isolates (tobramycin MIC range: 0.25–32 mg/L) and six *P. aeruginosa* isolates (tobramycin MIC range: 0.05 to >64 mg/L). The phenotypic and known genotypic profiles of these isolates are shown in Table 1 and have been reported previously.[6] Additionally, a *K. pneumoniae* isolate (CDC 831) served as an internal QC that was tested against previously described plasma and ELF HSRs of levofloxacin on each study day.[9] To demonstrate consistency with prior model experience, QC acceptance criteria for the model were based on the following: 24 h control growth of ≥1 $\log_{10}$ cfu/lung relative to the 0 h control; greater magnitude of bacterial reduction with the levofloxacin plasma HSR relative to the ELF HSR; and achievement of net killing with the plasma HSR.[9] In instances where any of the above criteria were not met, all data from the study run were discarded and experiments were subsequently repeated.

Sample size for *in vivo* efficacy studies was calculated as follows: (i) for typical antimicrobial agents, optimal dosing regimens usually produce approximately 2–3 $\log_{10}$ decrease in bacterial density with 40% coefficient of variation (CV); and (ii) in order to have an observed mean that deviates from the true mean by no more than 1 SD using a two-sided 95% CI with 80% probability, six datapoints are required.

Although the plasma and ELF HSRs for the respective drugs were determined and confirmed previously, additional efforts to characterize exposures concurrently with the *in vivo* efficacy runs were undertaken utilizing methodology used in the historic confirmations to ensure stability of the regimens in the model over time and among animals infected with different isolates.[10] In brief, at predefined timepoints, groups of mice receiving respective HSRs of meropenem, cefiderocol or tobramycin had blood sampled via retro-orbital bleeding (two timepoints, under anaesthesia with isoflurane 2%–3% v/v in 100% oxygen via inhalation) and/

Table 1. Phenotypic and known genotypic profiles of *K. pneumoniae* and *P. aeruginosa* isolates utilized during *in vivo* efficacy studies

			MIC (mg/L)			
	Isolate origin	Isolate ID	MEM	FDC	TOB	Known genotypic information
K. pneumoniae	CDC Bank	106	>64	8	>64	aac(3)-IId, aac(6′)-Ib, aadA1, aadA2, aph(3″)-Ib, aph(3′)-VI, aph(6)-Id, armA, ARR-2, ble-MBL, catB4, cmlA5, CTX-M-15, dfrA1, dfrA12, EMRD, ere(A), fosA5, KDEA, mph(E), msr(E), NDM-1, oqxA, oqxB20, OXA-1, OXA-9, qacEdelta1, sat2, SHV-28, sul1, sul2, TEM-1A
	CDC Bank	129	16	2	32	aac(6′)-Ib, aadA2, aph(3′)-Ia, catA1, dfrA12, KPC-3, mph(A), OmpK35, oqxA, oqxB, sul1, TEM-1A
	CDC Bank	160	8	0.125	0.25	fosA, oqxA, oqxB, OXA-48, SHV-11
	CDC Bank	504	8	0.25	8	aac(6′)-Ib-cr, catB4, CTX-M-15, OXA-48(c), SHV-OSBL(b), tet(A), tet(R)
	CDC Bank	523	64	2	8	aadA1, aph(3′)-Ia, dfrA1, KPC-2, sul1
	CDC Bank	542	2	>32	>64	aac(6′)-Ib, aadA2, catA1, dfrA12, EMRD, KDEA, mph(A), oqxA, oqxB, SHV-12, sul1
	CDC Bank	548	16	1	0.25	aph(3″)-Ib, aph(6)-Id, CTX-M-15, dfrA14, EMRD, fosA, KDEA, KPC-3, oqxA, oqxB20, QnrS1, SHV-28, sul2, TEM-1
	CDC Bank	555	>64	4	>64	aac(6′)-Ib-G, aadA1, aph(3′)-Ia, ARR-2, ble-MBL, catB, CTX-M-15, dfrA14, EMRD, KDEA, mph(A), NDM-5, oqxA, oqxB25, OXA-232, OXA-9, QnrS1, rmtF1, SHV-12, sul1, TEM-1A
	CDC Bank	560	>64	16	>64	aac(3)-IId, aac(6′)-Ib-AKT, aadA1, armA, ARR-2, ble-MBL, catA1, catB4, cmlA5, CMY-4, CTX-M-15, dfrA1, EMRD, ere(A), fosA, KDEA, mph(E), msr(E), NDM-1, oqxA, oqxB20, OXA-1, OXA-9, sat2, SHV-100, sul1, TEM-1A
	CDC Bank	831	≤0.063	0.5	8	aac(3)-IIa, aph(3′)-Ib, aph(6)-Id, catB4, CTX-M-15, dfrA14, OXA-1, QnrB1, SHV-187, sul2, TEM-1B, tet(A), tet(R)
	CDC Bank	848	64	4	>64	aac(3)-IIa, aac(6′)-Ib-cr, catB4, CTX-M-15, dfrA1, Omp35, OmpK35, oqxA, oqxB, OXA-1, OXA-48, QnrS1, SHV-11, sul1, TEM-1B, tet(A), tet(R)
	PEI	Kp C1.112	≤0.063	0.25	0.5	Unknown
	PEI	Kp C1.113	≤0.063	0.25	0.25	Unknown
	PEI	Kp C1.147	32	2	>64	Unknown
	PEI	Kp C1.151	>64	2	32	KPC
	DSMZ	30104	0.125	0.25	0.125	Unknown
P. aeruginosa	CDC Bank	459	16	0.063	2	OXA-50
	CDC Bank	767	>64	8	>64	GES-20
	CDC Bank	771	>64	1	>64	GES-19, GES-20
	PEI	Pa 88198	2	0.25	0.5	Unknown
	PEI	Pa 88276	1	0.5	1	Unknown
	PEI	Pa 88356	32	4	2	Unknown
	CAIRD	PSA INT 2-41	16	16	>64	aac(6′)-Ib, aac(6′)-Ib-cr, aadA6, aph(3′)-IIb, CTX-M-2, OXA-488, PDC-35, catB7, fosA, qacE, sul1
	CAIRD	PSA INT 4-99	8	8	>64	aac(6′)-Ib-cr, aadA6, aph(3′)-IIb, OXA-14, OXA-488, PDC-35, catB7, cml, cmlA1, fosA, qacE, sul1
	CAIRD	PSA INT 5-19	>64	0.125	>64	OXA-488, PAO (PDC-35), GES-5
	CAIRD	PSA INT 5-35	16	0.5	0.5	aph(3′)-IIb, OXA-488, PDC-471, catB7, fosA
	CAIRD	PSA INT 12-28	8	1	16	aac(6′)-Ib, aadA6, aph(3′′)-Ib, aph(3′)-IIb, aph(3′)-VIb, aph(3′)-XV, aph(6)-Id, GES-1, OXA-488, PDC-35, PER-1, catB7, fosA, qacE, sul1, tet(G)
	CAIRD	PSA US 4-27	64	16	>64	OXA-17/142, OXA-395, PAO (PDC-44), VIM-2

LVX, levofloxacin; MEM, meropenem; FDC, cefiderocol; TOB, tobramycin.

or cardiac puncture (one terminal timepoint). Proparacaine hydrochloride ophthalmic solution 0.5% was applied to the eye (1–2 drops) after blood sampling via retro-orbital bleeding. The volume of blood collected was 0.15 mL per sample via retro-orbital bleeding with subsequent fluid replacement using 0.2 mL normal saline given intraperitoneally. Mice were euthanized by CO_2 exposure prior to cardiac puncture. Following blood collection by cardiac puncture, but prior to cervical dislocation, bronchoalveolar lavage (BAL) fluid was collected from the mice at the same timepoints. A catheter was inserted into the trachea of the mice, and lungs were lavaged with four separate aliquots of 0.4 mL of normal saline. The BAL fluid was withdrawn immediately after injection and pooled for each animal. Pooled BAL was centrifuged for 10 min at 4°C and the supernatant was collected for analysis. Mice receiving plasma-based HSRs did not undergo BAL, and mice receiving ELF-based HSRs did not undergo retro-orbital bleeding.

Ethics

Animals were maintained and utilized in accordance with National Research Council recommendations. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital (Assurance #A3185-01).

Results

In vivo efficacy studies

Levofloxacin quality control

The QC isolate CDC 831 was tested against levofloxacin plasma and ELF HSRs during 27 separate *in vivo* efficacy experiments. The quantitative cfu/lung values from each study are presented in Figure S1 (available as Supplementary data at *JAC* Online). The data from four experiments were discarded because the QC failed to meet the prespecified criteria for acceptance as follows: two failed to reach 24 h control growth of ≥1 $\log_{10}$ cfu/lung relative to the 0 h control; one failed to achieve greater magnitude of bacterial reduction with the levofloxacin plasma HSR relative to the ELF HSR; and one failed to achieve net bacterial reduction with the plasma HSR.

Meropenem

Across all tested *K. pneumoniae* isolates, the mean ± SD $\log_{10}$ cfu/lung starting bacterial burden and 24 h net growth were 7.30 ± 0.16 and 1.77 ± 0.23, respectively. For *P. aeruginosa* isolates, they were 5.95 ± 0.31 and 3.05 ± 0.70 $\log_{10}$ cfu/lung, respectively. The 24 h change in $\log_{10}$ cfu/lung after receiving saline control, meropenem plasma HSR and meropenem ELF HSR for each isolate is presented in Figure 1.

Among *K. pneumoniae* isolates there was clear differentiation of activity between the three isolates with MICs of ≤2 mg/L (the CLSI intermediate breakpoint), and the seven isolates with MICs of >4 mg/L (the CLSI resistant breakpoint).[11] Resistant isolates all experienced net growth on both the meropenem plasma and ELF HSR, often similar to saline control, while intermediate and susceptible isolates experienced multilog$_{10}$ kill regardless of meropenem HSR. Conversely, both meropenem HSRs achieved ≥1 $\log_{10}$ kill in *P. aeruginosa* isolates with MICs of ≤16 mg/L. Both *P. aeruginosa* isolates with MICs of >64 mg/L demonstrated multilog$_{10}$ growth upon administration of the meropenem ELF HSR, but PSA INT 5-19 showed 1 $\log_{10}$ kill against the plasma HSR, whereas PSA 1866 grew similarly to the growth control.

Meropenem exposures quantified concurrently with *in vivo* efficacy studies were concordant with the original regimen confirmation studies in both plasma and ELF matrices. These data are presented graphically in Figure S2.

Cefiderocol

Among the 10 *K. pneumoniae* isolates tested against the cefiderocol HSRs, the mean ± SD $\log_{10}$ cfu/lung starting bacterial burden and 24 h net growth were 7.27 ± 0.12 and 1.66 ± 0.24,

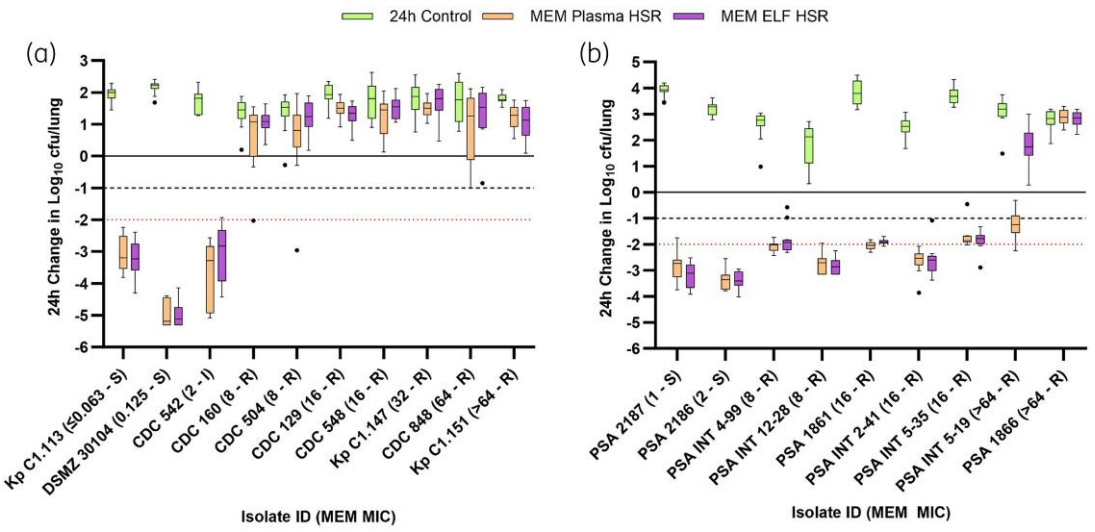

Figure 1. Quantitative cfu/lung data following administration of humanized meropenem (2 g every 8 h as 3 h infusion) plasma and ELF exposures in the COMBINE murine neutropenic pneumonia model against (a) *Klebsiella pneumoniae* and (b) *Pseudomonas aeruginosa* isolates. Boxes represent IQR, with horizontal line denoting the median. Whiskers determined by Tukey's test and outliers displayed as individual dots. Solid line denotes stasis. Dashed line denotes 1 $\log_{10}$ kill. Red dotted line denotes 2 $\log_{10}$ kill. S/I/R, susceptible/intermediate/resistant according to CLSI M100 Thirty-Fifth Edition.

respectively. For *P. aeruginosa* isolates they were 5.74 ± 0.25 and 3.58 ± 0.69 $\log_{10}$ cfu/lung, respectively. Individual isolate 24 h change in $\log_{10}$ cfu/lung after administration of saline control, cefiderocol plasma HSR and cefiderocol ELF HSR is shown in Figure 2.

The efficacy of the cefiderocol HSRs aligned well with the phenotypic profiles and existing CLSI breakpoints (S: ≤ 4 mg/L; I: 8 mg/L; R ≥ 16 mg/L) across both species.[11] All susceptible and intermediate isolates achieved ≥1 $\log_{10}$ kill with minimal differentiation in the magnitude of killing between the plasma and ELF HSRs. *K. pneumoniae* isolate CDC 560 (cefiderocol MIC = 16 mg/L) demonstrated 1 $\log_{10}$ kill with both cefiderocol HSRs. Differences in cfu reduction between the matrix HSRs were most pronounced among *P. aeruginosa* isolates with MICs of 16 mg/L (PSA INT 2-41 and PSA US 4-27) at the resistance breakpoint, although both resulted in net mean growth with considerable intermouse variability. *K. pneumoniae* isolate CDC 542 (cefiderocol MIC > 32 mg/L) grew similarly to untreated control.

Cefiderocol concentration-versus-time profiles from PK sampling taken during *in vivo* efficacy studies are shown in Figure S3. The ELF profile was well aligned with historic HSR determination, as was the plasma profile over the first 3 h, but there was some variance from historic determinations at the later observed timepoints.

Tobramycin

K. pneumoniae and *P. aeruginosa* isolates examined in the COMBINE murine neutropenic pneumonia model against humanized plasma and ELF exposures of tobramycin displayed similar mean ± SD $\log_{10}$ cfu/lung starting bacterial burden and 24 h net growth to those tested against meropenem and cefiderocol—7.33 ± 0.29 and 1.71 ± 0.27 $\log_{10}$ cfu/lung, respectively, for *K. pneumoniae*, and 6.23 ± 0.12 and 3.00 ± 0.74 for *P. aeruginosa*. Figure 3 contains individual isolate data for untreated controls and tobramycin plasma and ELF HSRs.

Tobramycin HSR efficacy in the model was harmonious with clinical breakpoints for both *K. pneumonia* (S: ≤2 mg/L; I: 4 mg/L; R ≥ 8 mg/L) and *P. aeruginosa* (S: ≤1 mg/L; I: 2 mg/L; R ≥ 4 mg/L) isolates.[11] Susceptible isolates demonstrated multilog$_{10}$ killing over 24 h after administration of the HSR associated with either matrix. The *P. aeruginosa* isolate at the intermediate breakpoint (CDC 459—tobramycin MIC = 2 mg/L) showed extensive variability but net growth. All resistant isolates failed to achieve ≥1 $\log_{10}$ of kill with either tobramycin HSR, with *P. aeruginosa* isolates growing similarly to untreated controls, and *K. pneumoniae* isolates demonstrating net growth but numerically lower cfu/lung values at 24 h compared with saline control.

Tobramycin plasma exposures were quantified in mice receiving the plasma HSR on *in vivo* efficacy study days (Figure S4). Observed concentrations reasonably recapitulated the historic plasma HSR regimen confirmation. Similarly, BAL samples were collected in mice receiving the ELF HSR, but inconsistencies in the analytical outputs for these samples precluded their reporting as reanalysis of individual samples were unable to reproduce concentrations within 20% of the original, and sample volume was depleted.

Discussion

These data describe the quantitative cfu/lung performance of humanized plasma and ELF exposures of meropenem, cefiderocol and tobramycin against a challenge set of *K. pneumoniae* and *P. aeruginosa* in the COMBINE murine neutropenic pneumonia model. This manuscript is the last in a series of four manuscripts to: establish a diverse pheno-genotypic challenge set of *K. pneumoniae* and *P. aeruginosa* suitable for use in the COMBINE murine

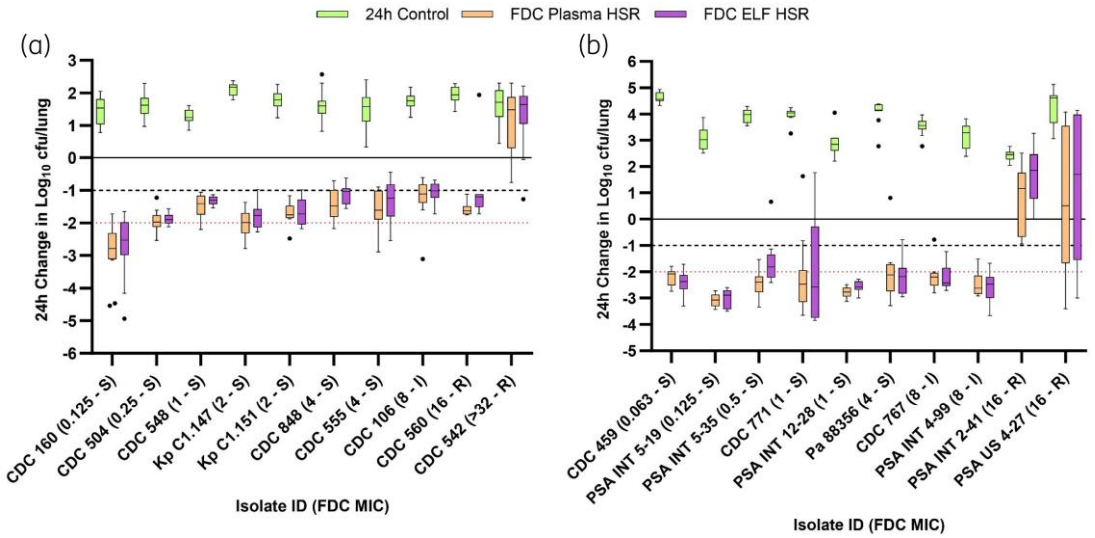

Figure 2. Quantitative cfu/lung data following administration of humanized cefiderocol (2 g every 8 h as 3 h infusion) plasma and ELF exposures in the COMBINE murine neutropenic pneumonia model against (a) *Klebsiella pneumoniae* and (b) *Pseudomonas aeruginosa* isolates. Boxes represent IQR, with horizontal line denoting the median. Whiskers determined by Tukey's test and outliers displayed as individual dots. Solid line denotes stasis. Dashed line denotes 1 $\log_{10}$ kill. Red dotted line denotes 2 $\log_{10}$ kill. S/I/R, susceptible/intermediate/resistant according to CLSI M100 Thirty-Fifth Edition.

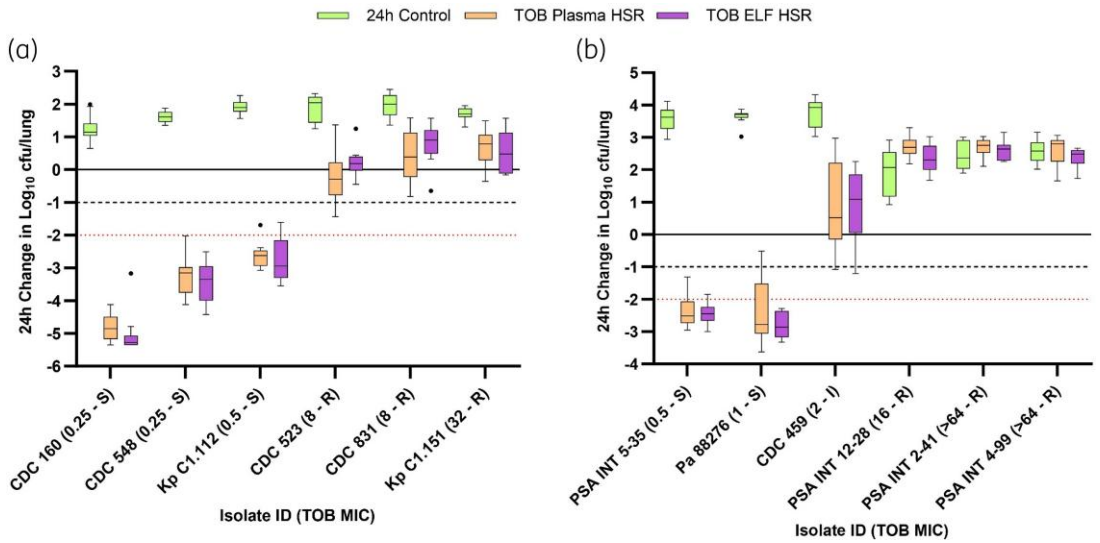

Figure 3. Quantitative cfu/lung data following administration of humanized tobramycin (7 mg/kg as 30 min infusion) plasma and ELF exposures in the COMBINE murine neutropenic pneumonia model against (a) *Klebsiella pneumoniae* and (b) *Pseudomonas aeruginosa* isolates. Boxes represent IQR, with horizontal line denoting the median. Whiskers determined by Tukey's test and outliers displayed as individual dots. Solid line denotes stasis. Dashed line denotes 1 $\log_{10}$ kill. Red dotted line denotes 2 $\log_{10}$ kill. S/I/R, susceptible/intermediate/resistant according to CLSI M100 Thirty-Fifth Edition.

neutropenic pneumonia model; develop and confirm HSRs in both plasma and ELF matrices for tigecycline, levofloxacin, meropenem, cefiderocol and tobramycin; and define quantitative cfu/lung benchmarks against this isolate challenge set after administration of each matrix HSR for the respective compounds.[6,9,10]

The standardization of multiple facets of preclinical animal infection models has the potential to provide benefit and clarity to the development space. The COMBINE murine neutropenic pneumonia model protocol defines specifications for many of the most essential model elements while still allowing enough flexibility for existing laboratories to maintain many of their existing workflows and techniques. Therefore, a shift towards general acceptance of this protocol should be minimally disruptive to research laboratories, especially given that bacterial isolates with defined phenotypic and genotypic profiles that reproducibly induce infection under the stipulated constructs of the COMBINE protocol have already been defined.[6] Importantly, isolates other than those sourced from the CDC Bank will be made readily available to investigators through the DSMZ. While these efforts are a significant first step towards global standardization of preclinical animal infection models, it should be noted that they incorporate only two bacterial species and only one specific infection model. The preclinical infectious disease research community could additionally benefit from similar analyses with *Acinetobacter baumannii* in this model, and the consensus on standardized protocols for other infection models like the murine neutropenic thigh infection model.

Meropenem, cefiderocol and tobramycin have well-defined PK/PD targets based on plasma exposures.[12–14] The purpose of our current study was to establish quantitative bacterial density benchmarks after exposures to humanized plasma and ELF profiles and not to re-define PK/PD targets for these compounds. However, existing clinical breakpoints heavily weigh PK/PD targets in the context of PTA at a given dose, which allows for clinical back-translation of these HSRs in terms of expected efficacy. In general, there was strong agreement between clinical breakpoints and efficacy of humanized exposures across all three tested antibiotics, which represents a validation of established PK/PD targets and the translational relevance of the COMBINE murine neutropenic lung infection model. Perhaps the starkest contrast was the different activity of the meropenem HSRs between *K. pneumoniae* and *P. aeruginosa*. *K. pneumoniae* isolates with meropenem MICs of 8 and 16 mg/L uniformly demonstrated net growth on meropenem HSRs, whereas *P. aeruginosa* isolates with the same meropenem phenotype were able to achieve >1 $\log_{10}$ of kill. Based on free plasma exposures (on which PK/PD targets are often derived), it would not be unreasonable to expect this activity in *P. aeruginosa* at MICs up to 16 mg/L based on the PK/PD target achievement of >40% $fT_{>16\ mg/L}$ with an optimized meropenem dose of 2 g q8h as a 3 h extended infusion. However, recent efforts have shown that phenotype alone may be insufficient to predict antibiotic efficacy, especially for β-lactams when carbapenemases are present.[15,16] Underlying mechanisms of meropenem resistance between *K. pneumoniae* and *P. aeruginosa* are often quite different, with Enterobacterales being driven by carbapenemases and *P. aeruginosa* mainly through porin and efflux pump regulation.[17] Indeed, all tested *K. pneumoniae* with meropenem MICs of 8 or 16 mg/L harboured a carbapenemase (either KPC or OXA-48), whereas no carbapenemases were present in the *P. aeruginosa* isolates with the same meropenem MICs. This alone may be sufficient to explain the differentiation in activity between the two bacterial species when administered equivalent humanized exposures of meropenem. Nonetheless, as previously reported, it should be noted that the starting bacterial burden of *K. pneumoniae* isolates were uniformly targeted 1 $\log_{10}$ higher relative to *P. aeruginosa* isolates in the COMBINE model in order to achieve viability.[6] This

difference could plausibly influence antibiotic efficacy but was not noted with either cefiderocol or tobramycin.

Throughout this four-part series we have belaboured the point of quantifying drug exposures at the target site of infection in preclinical animal models to improve bench-to-bedside translation because interspecies differences in penetration are common. As described in the development and confirmation of the HSRs for meropenem, cefiderocol and tobramycin, these three drugs were no exception.[10] As the magnitude of penetration varied by ~30%–50% between mouse and man, it would not be expected for the efficacy of the plasma and ELF HSRs to be different for every isolate. In fact, only isolates on the slippery slope of PK/PD attainment at or near the breakpoint are likely to be impacted. Differences in penetration of this magnitude would be unlikely to sway target exposure attainment for isolates that are either exquisitely susceptible or highly resistant. Indeed, with few exceptions this is what our dataset demonstrated.

Modern PK/PD preclinical animal model assessments generally determine PK in infected animals up front and then assume that these exposures are consistent with those attained during *in vivo* efficacy studies, without actual reconfirmation of exposures. In general, this is a very reasonable assumption as the reconfirmation of exposures concurrently with *in vivo* efficacy studies requires a notable level of effort and resources. Although identical exposures are not expected to be achieved *in vivo* over a large series of experiments as we have conducted herein due to the intra-individuality of PK in the mice, observed variability during treatment is expected to encompass that which has been originally defined in the HSR regimens, as well as the increases seen in human PK studies of infected patients. Indeed, as shown in the Supplementary data, we demonstrated remarkable PK consistency between the original HSRs and the profiles obtained during the *in vivo* efficacy studies for meropenem in both matrixes, and cefiderocol in the ELF. Reasonably expected variation from the original HSRs was observed for both tobramycin and cefiderocol plasma. Having demonstrated variability within our own laboratory, it is highly encouraged that confirmatory PK studies be undertaken prior to future use of our mg/kg dosing schemes as differences in the drug products (i.e. pharmaceutical versus analytical grade), formulations and manufacturers, as well as mice vendors may result in considerably different drug exposures in each of the biological matrixes.

An additional strength of these investigations was the inclusion of an internal QC with every *in vivo* efficacy assessment. Similar to the reconfirmation of drug exposures, this practice is not commonplace for PK/PD animal infection models for many of the same reasons. However, it should be acknowledged that these models are highly complex biological systems, with many moving parts and both internal and external variables. While all experiments are well intentioned, it may not be possible to identify when one of the variables, whether it be previously understood or currently unrecognized, has been changed or manipulated. To this end, the addition of a QC can help demonstrate model stability and reassure that any unknowing changes in study variables are of limited impact. Of the 27 experiments undertaken, there were 4 instances (15%) in which the QC demonstrated unexpectedly discordant results. Inclusion of this QC isolate in each investigational run revealed a high level (85%) of model reproducibility over the course of months of repetitive studies, while also serving to identify experimental inconsistencies that might have otherwise been missed when using different isolates from run to run. Although not typically done for conventional PD profiling studies, inclusion of the QC isolate confirmed the robustness of the experimental model, as well as the suitability and applicability of the defined bacterial density benchmarks determined for the drug–bug combination studies.

In summary, we have (i) established a diverse phenotypic and genotypic challenge set of *K. pneumoniae* and *P. aeruginosa* suitable for use in the COMBINE murine neutropenic pneumonia model, (ii) developed and confirmed HSRs in both plasma and ELF biological matrixes for tigecycline, levofloxacin, meropenem, cefiderocol and tobramycin and (iii) now have set quantitative cfu/lung benchmarks using both plasma and ELF HSRs with all five compounds against the isolate challenge set. This dataset and the use of the COMBINE protocol serves to further minimize experimental inconsistency and improve the interpretability of endpoint data such as quantitative bacterial density among different laboratories. Moreover, this collective series of studies[6,9,10] supported by CARB-X has broad utility for the development of novel therapies (i.e. small molecules, phage, lysins) because these investigations provide translational PK and PD benchmarks for an array of antibacterials previously demonstrated to be effective in the treatment of infection in man.

Acknowledgements

These data were presented in part at IDWeek 2024 (Los Angeles, CA, USA). We would like to thank: Gabrielle Barocsi, Ashley Boudreau, Kelly Bradley, Taylor Cardin, Connor Chung, Janice Cunningham, Aliaa Fouad PhD, Brianna Fraser, Ashley Gonzalez, Christina Konig PhD, Mia Leo, Cole McGrath, Wendylee Rodriguez, Debora Santini, Rebecca Stewart, Christina Sutherland and Jennifer Tabor-Rennie from the Center for Anti-Infective Research and Development for their vital assistance in the conduct of this study.

Funding

This study was funded by CARB-X (Boston, MA, USA). CARB-X is funded in part with federal funds from the U.S. Department of Health and Human Services; Administration for Strategic Preparedness and Response; Biomedical Advanced Research and Development Authority; under agreement number: 75A50122C00028. CARB-X is also funded by awards from Wellcome (WT224842), Germany's Federal Ministry of Education and Research (BMBF), the UK Department of Health and Social Care as part of the Global Antimicrobial Resistance Innovation Fund (GAMRIF), the Public Health Agency of Canada (PHAC), the Bill & Melinda Gates Foundation and the Novo Nordisk Foundation. The U.S. National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) in HHS, provides support in the form of in-kind services through access to a suite of preclinical services for product development. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any CARB-X funders.

Transparency declarations

A.J.F. and A.M.P. have none to declare. E.M.D. is an employee of the study funder, CARB-X. D.P.N. is a consultant, speaker bureau member or has

received research funding from: AbbVie, CARB-X, Cepheid, Innoviva, Merck, Pfizer and Shionogi.

Supplementary data

Figures S1 to S4 are available as Supplementary data at *JAC* Online.

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