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In vivo efficacy & resistance prevention of cefiderocol in combination with ceftazidime/avibactam, ampicillin/sulbactam or meropenem using human-simulated regimens versus *Acinetobacter baumannii*

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Objective: Evaluate the *in vivo* efficacy and resistance prevention of cefiderocol in combination with ceftazidime/avibactam, ampicillin/sulbactam and meropenem using human-simulated regimens (HSR) in the murine infection model.

Methods: In total, 15 clinical *A. baumannii* were assessed: cefiderocol MICs, 2 mg/L (previously developed resistance on therapy), $n=3$; 8 mg/L, $n=2$; ≥32 mg/L, $n=10$ (including VEB and PER-harbouring isolates). Mice received inactive control, cefiderocol, cefiderocol + ceftazidime/avibactam (C-CZA), cefiderocol + ampicillin/sulbactam (C-SAM) or cefiderocol + meropenem (C-MEM) HSRs. The mean change in $\log_{10}$ cfu/thigh compared with starting inoculum was assessed. Resistance development on treatment was a >4-fold increase in MIC relative control animals. *In vitro* activities of combinations were assessed by disc stacking.

Results: Against cefiderocol-non-susceptible isolates, combinations produced significant kill with C-CZA -3.75 ± 0.37 reduction in $\log_{10}$ cfu/thigh, C-SAM produced -3.55 ± 0.50 and C-MEM produced -2.18 ± 1.75 relative to baseline. Elevated MICs in cefiderocol treated animals occurred in three out of three isolates with MICs of 2 mg/L. Of these isolates, one developed elevated MICs with C-MEM compared with none treated with C-CZA or C-SAM. Disc stacking with C-CZA or C-SAM returned all isolates to at least the CLSI intermediate breakpoint, which may correlate with *in vivo* efficacy.

Conclusions: Against cefiderocol-non-susceptible isolates, cefiderocol + ceftazidime/avibactam or ampicillin/sulbactam HSR produced *in vivo* kill against all 12 cefiderocol-non-susceptible isolates. Cefiderocol with ceftazidime/avibactam or ampicillin/sulbactam prevented the development of resistance during treatment against cefiderocol-high-end-susceptible isolates with a propensity for resistance on therapy. These data support the clinical evaluation of cefiderocol with ceftazidime/avibactam or ampicillin/sulbactam against *A. baumannii*, including multi-drug-resistant isolates.

Background

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) remains a clinical challenge as more than 57 000 deaths worldwide were attributed to this pathogen in 2019.[1] Infection that is multi-drug-resistant and with CRAB has been associated with higher inpatient mortality in patients with *A. baumannii* infections compared to those with infections caused by susceptible isolates necessitating novel therapeutic strategies.[2] Combination therapy for *A. baumannii* has been studied in numerous clinical trials although results have been inconclusive because of the heterogeneous populations and combinations studied largely consisted of polymyxin-based regimens that are plagued by high toxicity and a poor correlation with efficacy.[3–5] Contemporary guidance recommend the combination of two active antimicrobials for moderate to severe infections caused by CRAB.[6] Unfortunately,

resistance to carbapenems is accompanied by cross-resistance to other β-lactams as well as agents from different antimicrobial classes necessitating novel agents and combinations.[7]

Due to its novel siderophore mechanism of cell entry and stability against acquired/intrinsic β-lactamases, cefiderocol is active *in vitro* against *A. baumannii* including CRAB.[8] Indeed, cefiderocol inhibited 90% of *A. baumannii* isolates at an MIC of ≤1 mg/L despite meropenem-non-susceptibility.[9] We previously determined the *in vivo* efficacy of humanized cefiderocol exposures against clinical *A baumannii* where >1 $\log_{10}$ kill was observed at 72 h in five out of seven isolates with MICs up to 2 mg/L.[10] Development of resistance on therapy was observed although rare.[10] A randomized controlled trial in nosocomial pneumonia has determined the efficacy of cefiderocol against *A. baumannii* including carbapenem-resistant strains, although a numeric difference in all-cause mortality was noted in the CREDIBLE-CR study in patients with CRAB infections where mortality was 49% in cefiderocol treated patients compared with 18% in best available therapy warranting further investigation.[11,12] Despite the potent *in vitro* and *in vivo* activity, isolates with elevated MICs have been described, notably *A. baumannii* harbouring PER and VEB-β-lactamases, further reducing the available treatment options.[13] The addition of β-lactamase inhibitors (i.e. avibactam) to cefiderocol have reduced cefiderocol MICs against cefiderocol-non-susceptible *A. baumannii* including isolates with and without acquired β-lactamases (i.e. VEB and PER).[13,14] The novel mechanism and *in vitro* potency make cefiderocol an attractive component of combination therapy warranting investigation.

In vitro modelling has demonstrated synergy of cefiderocol when administered with ceftazidime/avibactam, ampicillin/sulbactam, meropenem or amikacin against *A. baumannii*.[15] The present study aimed to evaluate the *in vivo* efficacy of cefiderocol in combination with ceftazidime/avibactam, ampicillin/sulbactam and meropenem at humanized exposures over 72 h against cefiderocol-non-susceptible (MIC 8–>32 mg/L) *A. baumannii*. The same combinations were evaluated against cefiderocol-high-end-susceptible isolates (MIC 2 mg/L) to evaluate whether combination therapy prevented the development of *in vivo* resistance. *In vivo* activity in the model may translate to clinical efficacy as the combinations evaluated are at clinically relevant exposures. An evaluation of practical *in vitro* testing using stacking of disc diffusion to correlate with the *in vivo* efficacy may help guide therapeutic selections of these combinations in the clinic against *A. baumannii* isolates where treatment options are currently limited.

Materials and methods

Ethics

The present study was reviewed and approved by the Institutional Animal Care and Use Committee of Hartford Hospital. All experiments were conducted in alignment with the National Research Council of the National Academy of Sciences standards.

Antimicrobial test agents

Commercially available cefiderocol (Shionogi, Japan), ceftazidime (Sandoz, IL, USA; Astral Steri Tech Pvt Ltd, India), ampicillin/sulbactam (Meitheal Pharmaceuticals, IL, USA) and meropenem (Aurobindo Pharma Limited-Hyderabad, India) were used for all *in vivo* experiments. Analytical grade avibactam (MedChemExpress, NJ, USA) was used in the ceftazidime/avibactam human simulating regimen (HSR).[16] Analytical standard powders were used for the preparation of broth microdilution MIC trays (Cefiderocol: Shionogi, Japan; ceftazidime: MedChemExpress, NJ, USA; avibactam: MedChemExpress, NJ, USA; meropenem: Sigma-Aldrich, WY, USA; ampicillin: MedChemExpress, NJ, USA; sulbactam: United States Pharmacopeial Convention, MD, USA).

Isolates

Fifteen *A. baumannii* clinical isolates were tested in the murine model. Table 1 describes the modal MICs for each isolate to cefiderocol, ceftazidime/avibactam, ampicillin/sulbactam (MIC reported as sulbactam component) and meropenem in triplicate. Available genotypic data are presented in Table 1. Three cefiderocol-high-end-susceptible isolates (MIC = 2 mg/L) were chosen to evaluate the use of combination therapy to prevent the development of resistance as they have been previously found to have increased MICs in the model post-exposure.[10,17] The remaining 12 isolates were selected due to baseline cefiderocol-non-susceptibility to evaluate the *in vivo* pharmacodynamics of the combinations against highly resistant isolates.

In vitro MIC testing

Pre- and post-exposure MICs were conducted for cefiderocol in iron-depleted CAMHB per CLSI Standards as previously described.[10,18,19]

To evaluate a clinically implementable *in vitro* combination testing, disc diffusion using conventional antimicrobial susceptibility discs stacked one agent on top of the other were assessed for each combination. Briefly, inocula were prepared per CLSI standards and the bacterial suspension was lawned onto Muller–Hinton Agar plates (Becton Dickenson, Franklin Lakes, NJ, USA). Cefiderocol discs (Hardy Diagnostics, Santa Maria, CA, USA) were then placed on the agar. The second agent disc was placed on top of the cefiderocol disc and 30 µL of saline was placed on top of the second disc. Plates were incubated per CLSI guidance and the zone of inhibition was read by qualified personnel. Combination MICs were conducted in iron-depleted CAMHB using the following combinations: cefiderocol with ceftazidime/avibactam (1:1 ratio for cefiderocol to ceftazidime, avibactam fixed at 4 mg/L), cefiderocol with ampicillin/sulbactam (1:2:1 ratio cefiderocol, ampicillin, sulbactam) and cefiderocol with meropenem (1:1 ratio). Inoculua were prepared and trays incubated per CLSI standards for *A. baumannii*.[18,19] The 1:1 ratio method was used since disc diffusion is validated against standard broth microdilution. As commercially available discs were used for ease of implementation clinically, the ratio of drugs in the disc is not altered, thus we compared the disc stacking to the MIC determined with an increasing ratio of the antibiotic, to which individual discs would be compared when tested alone because there is no gold standard methodology for *in vitro* combination testing.

Animals

Specific-pathogen-free, CD-1 mice (female, 20–22 g) were used for all *in vivo* experiments (Charles River Laboratories, Inc., Raleigh, NC, USA). Mice were acclimatized and housed as previously described.[20]

Neutropenic murine thigh infection model

Before all *in vivo* experiments, mice were pretreated with cyclophosphamide (150 mg/kg on day −4, and 100 mg/kg on day −1) and uranyl nitrate (5 mg/kg on day −3) via intraperitoneal injection. On the day of the experiment, one thigh per mouse was inoculated with a 0.1 mL injection intramuscularly of ~1×10^7 cfu/mL bacterial suspension. Antibiotic dosing commenced 2 h post-inoculation to allow the bacteria to reach log-phase growth.

Previously defined human-simulated doses of cefiderocol, ceftazidime/avibactam and meropenem were administered as 0.1 mL of

Table 1. Clinical *A. baumannii* isolates included the *in vivo* model

Isolate	Genotype	CFDC MIC	CZA MIC[a]	MEM MIC	SAM MIC
AB 147	OXA-23-like (PCR)	2	>64	64	32
AB 230	ADC-33, OXA-82	2	>64	32	4
AB 237	ADC, OXA-58-like	2	>64	16	8
AB 97	PER-1, OXA-58, ADC-76, OXA-68	8	>64	4	16
AB 319	ADC-Type, OXA-829, OXA-24	8	>16	>16	8
AB 318	ADC-33 (V317G variant), OXA-23, OXA-82	32	>16	>16	16
AB 320	ADC-33 (V317G variant); OXA-23, OXA-82	32	>16	>16	8
AB 313	PER-1, ADC-25, OXA-23, OXA-66	>32	64	64	32
AB 314	PER-1, ADC-11, OXA-66, OXA-72	>32	64	64	32
AB 316	ADC-25-like, OXA-66, OXA-72, PER-1, TEM-1D	>32	32	>64	32
AB 323	ADC-25-like; OXA-172	>32	>64	16	16
AB 324	ADC-11; OXA-66; OXA-72; PER-1; TEM-1D	>32	>16	>16	64
AB 325	ADC-25-like; OXA-9; OXA-24; OXA-51-like; TEM-1A; VEB-9	>32	16	>64	32
AB 326	ADC-25-like; OXA-82	>32	>64	16	2
AB 327	ADC-25-like; OXA-66; OXA-72; PER-13	>32	>64	64	8

CFDC = cefiderocol, CZA = ceftazidime/avibactam, MEM = meropenem, SAM = ampicillin/sulbactam
[a]Avibactam concentration fixed at 4 mg/L.

subcutaneous injections.[16,21] The murine sulbactam (administered with ampicillin) HSR was developed for the neutropenic murine thigh infection model. The free-sulbactam plasma concentration in the mice mimicked the plasma pharmacodynamic profile (free-time above MIC, free C_{max} and free-AUC) achieved in healthy volunteers treated with 3 g IV q8h as a 4 h infusion using previously established pharmacokinetic parameters from mice and humans.[6,22,23] Once defined, the murine free plasma profile of the HSR was reassessed. Confirmatory pharmacokinetic studies after the administration of sulbactam (with ampicillin) at 10, 12 and 7.5 mg/kg at 0, 1.5 and 3 h, respectively (repeated ever 8 h), produced observed concentrations similar to the murine predicted and human-simulated profiles (Table S1, Figure S1, available as Supplementary data at *JAC* Online).

In vivo efficacy studies

Groups of six mice were randomized to the following groups for each isolate: 0 h control (baseline bacterial burden), 72 h control (growth control), cefiderocol HSR, cefiderocol with ceftazidime/avibactam HSR, cefiderocol with ampicillin/sulbactam HSR and cefiderocol with meropenem HSR. The 72 h control and each antibiotic treatment group were administered for the 72 h experiment and aseptically harvested at 72 h for cfu enumeration. If the animal was moribund (i.e. unable to right themselves) they were sacrificed. Any animal sacrificed or that had succumbed to infection before the end of the 72 h study had their infected thigh harvested for cfu enumeration at that time.

In vivo efficacy was assessed as $\log_{10}$ change in cfu/thigh in 72 h control and treatment groups from the 0-h control (baseline bacterial burden). Efficacy was assessed using the translational endpoint of >1-$\log_{10}$ bacterial kill from baseline.[24] Statistical analysis was conducted using a one-way ANOVA with Tukey's *post hoc* test to determine between-group differences. As a proof of concept, five isolates were selected to assess the *in vivo* effects of meropenem, ceftazidime/avibactam and ampicillin/sulbactam to isolates with various MICs to establish the individual agents were active against susceptible isolates.

Post-exposure development of resistance was assessed in the cefiderocol-high-end-susceptible isolates. Development of resistance was defined as a >4× MIC dilution increase in the post-exposure MIC in a treatment group compared with the untreated 72-h controls.

Results

In vivo efficacy studies

All test isolates adequately established infection in the model with a mean baseline bacterial burden of 5.67 ± 0.68 $\log_{10}$ cfu/thigh across the 15 isolates that increased by a mean of 3.34 ± 0.62 $\log_{10}$ cfu/thigh in the untreated 72-h control groups.

As a proof of concept, five isolates were assessed *in vivo* over 72 h of treatment with sulbactam (with ampicillin) HSR, ceftazidime/avibactam HSR and meropenem HSR. The sulbactam HSR resulted in bacterial kill consistent with the MIC and exposure of the high-dose, extended-infusion HSR. Ceftazidime/avibactam resulted in variable activity while meropenem HSR activity was consistent with the *in vitro* MIC and both agents lacked appreciable activity when the MIC was elevated (Figure S2).

Twelve cefiderocol-non-susceptible isolates were assessed *in vivo* (cefiderocol MICs ranged 8–>32 mg/L). As predicted by the *in vitro* MICs, cefiderocol HSR treatment resulted in multilog bacterial growth in 11/12 isolates while kill was observed in one isolate at an MIC of 8 mg/L. On treatment with cefiderocol in combination with ceftazidime/avibactam or sulbactam (with ampicillin), 12/12 cefiderocol-non-susceptible isolates resulted in greater than 1-$\log_{10}$ kill (Figure 1) with a range of mean change in $\log_{10}$ cfu/thigh of −3.30 to −4.43 and −2.77 to −3.95 for each combination, respectively (Table S2). Conversely, cefiderocol in combination with meropenem resulted in 1-$\log_{10}$ kill in 9/12 isolates. Indeed, one isolate reached 1-$\log_{10}$ kill but was not significantly improved activity over cefiderocol alone (AB 319).

Three cefiderocol-high-end-susceptible isolates were tested to assess the development of resistance *in vivo*. Considering the pharmacodynamic study, cefiderocol HSR resulted in bacterial kill against all three isolates with a range of mean kill of −1.00 to −2.41 $\log_{10}$ cfu/thigh relative to baseline. Combination therapy significantly increased bacterial kill in three out of three isolates for cefiderocol plus ceftazidime/avibactam or ampicillin/sulbactam

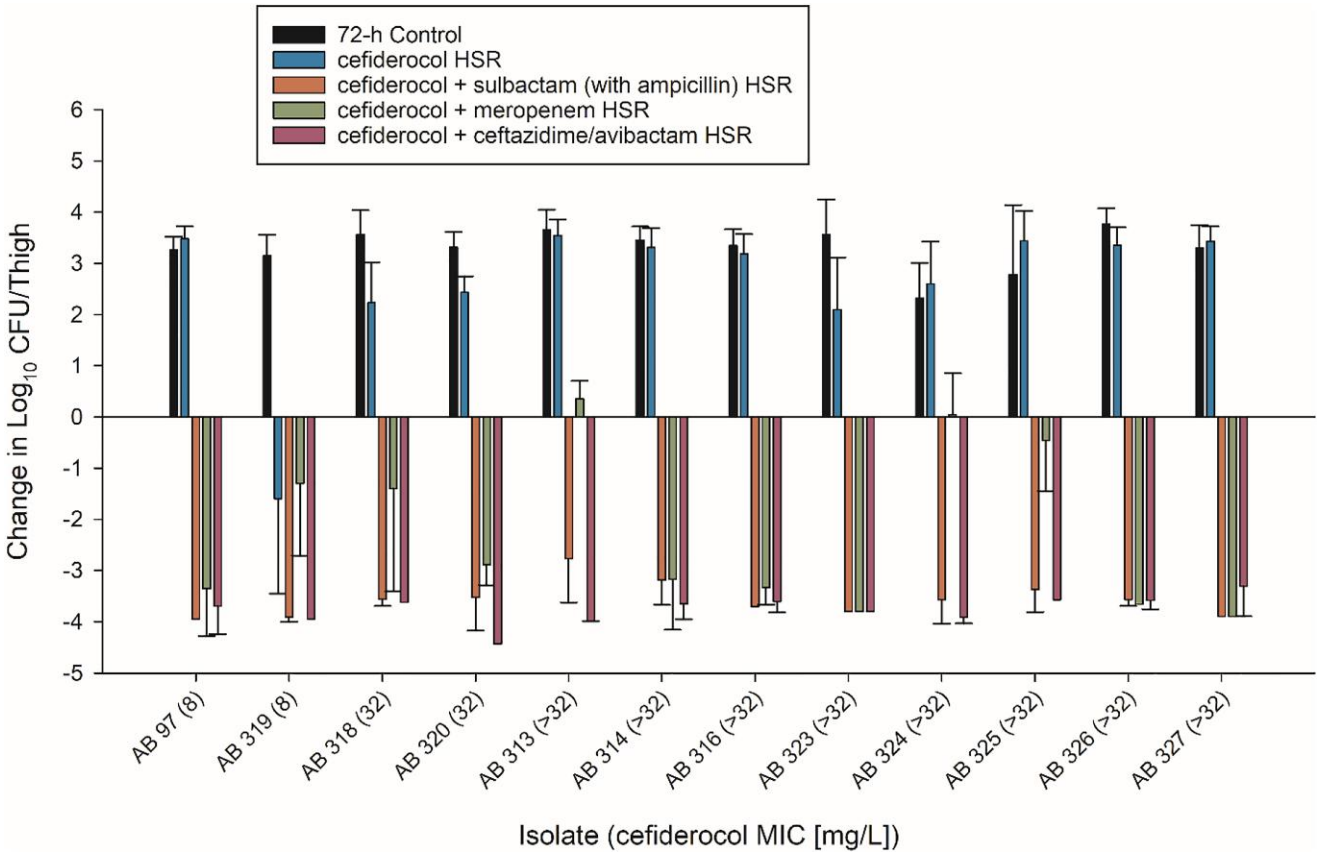

Figure 1. *In vivo* efficacy of humanized regimens of cefiderocol alone and in combination with ceftazidime/avibactam, ampicillin/sulbactam and meropenem against cefiderocol non-susceptible *A. baumannii* after 72 h of treatment.

and two out of three isolates for cefiderocol plus meropenem (Figure 2).

Post-exposure development of resistance

In cefiderocol HSR treated mice, post-exposure resistance developed in 8, 33 and 11% of thighs for AB 147, AB 230 and AB 237, respectively. All three combinations prevented the development of resistance for isolates AB 237 and AB 230 as no thighs exhibited resistance development to cefiderocol during combination therapy. For AB 147, 8% of thighs treated with cefiderocol and meropenem HSR developed elevated MICs on treatment while cefiderocol plus ceftazidime/avibactam or sulbactam (with ampicillin) resulted in no samples with elevated MICs.

In vitro combination testing

Broth microdilution modal MICs for cefiderocol with ceftazidime/avibactam, ampicillin/sulbactam and meropenem with a range of MICs of 0.06–4, 1–8 and 1–>32 mg/L for each agent, respectively. Tables S3 and S4 describe the modal broth microdilution MIC and the zone of inhibition for all replicates of the disc diffusion with each agent alone (Table S3) and stacked discs (Table S4). For the cefiderocol alone, seven isolates evaluated had microcolonies within the zone of inhibition for all replicates of the disc diffusion. When comparing the zone of inhibition of the stacked discs to the current cefiderocol susceptibility breakpoints per CLSI, 11/12 isolates tested with cefiderocol and ceftazidime/avibactam resulted in all zones of inhibition returning to the susceptible range with the single isolate not meeting these criteria returning to the intermediate range. For ampicillin/sulbactam, 9/12 reached the susceptible range while the remaining three reached at least the intermediate range for both cefiderocol and ampicillin/sulbactam. Interestingly, in the isolates that had microcolonies for cefiderocol alone, microcolonies within the zone of inhibition were dramatically reduced in the stacked disc experiments occurring in one replicate of one isolate for cefiderocol with ceftazidime/avibactam and six replicates for four different isolates for cefiderocol with ampicillin/sulbactam (one replicate for $n=3$ isolates and all three replicates for $n=1$ isolate). Similar to the *in vivo* findings, meropenem did not consistently return the zone of inhibition to either of the criteria with 9/12 isolates remaining resistant in the disc stacking experiments.

To evaluate cut-offs for the observed *in vivo* efficacy of the combinations, Figure 3 categorizes isolates by the modal combination MIC, the zone of inhibition of stacked discs (relative to current CLSI cefiderocol breakpoints) and the demonstration of >1-$\log_{10}$ kill. For both cefiderocol with ceftazidime/avibactam and cefiderocol with ampicillin/sulbactam, all 12 isolates had the zone of inhibition returned to at least intermediate range and resulted in >1-$\log_{10}$ kill.

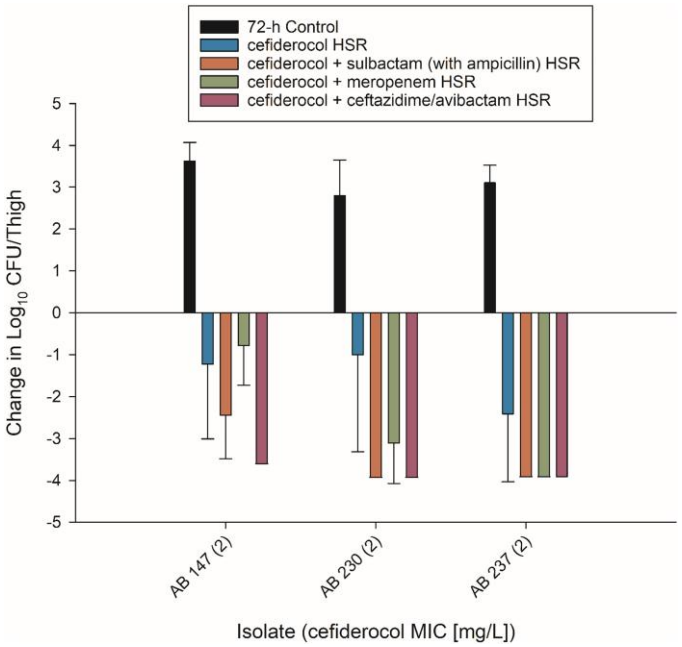

Figure 2. *In vivo* efficacy of humanized regimens of cefiderocol alone and in combination with ceftazidime/avibactam, ampicillin/sulbactam and meropenem against cefiderocol-susceptible *A. baumannii* after 72 h of treatment.

For meropenem, there was no clear relationship between zone size of the stacked disc experiment and *in vivo* efficacy but notably, all three isolates that failed to reach >1-$\log_{10}$ kill had stacked disc zone of inhibitions that remained in the resistant range. Based on the *in vitro* and *in vivo* findings a cut-off of returning the zone of inhibition using disc stacking to the intermediate range for cefiderocol with ceftazidime/avibactam or ampicillin/sulbactam may be a potential surrogate for *in vivo* efficacy.

Discussion

Although combination therapy is advocated for the treatment of carbapenem-resistant *A. baumannii*, the clinical evidence for this recommendation is controversial. The present study provides pre-clinical evidence of the microbiologic activity of cefiderocol in combination with ceftazidime/avibactam and sulbactam (with ampicillin) against clinical *A. baumannii* isolates with high-end-susceptible (MIC 2 mg/L) or non-susceptible (MIC 8–>32 mg/L) cefiderocol MICs using pharmacokinetic exposures similar to those seen in humans. Conversely, *in vivo* efficacy of the cefiderocol with meropenem combination was more variable. By using exposures of each agent that mimicked those seen in humans receiving each agent, these data provide a translational bridge to support the clinical evaluation of these rationally designed combinations.

Twelve clinical cefiderocol-non-susceptible *A. baumannii* isolates were tested to assess the *in vivo* efficacy of each combination. All isolates were meropenem-non-susceptible and 11 were sulbactam-non-susceptible, thus representing challenging clinical isolates. Consistent with the *in vitro* MICs, cefiderocol HSR monotherapy lead to *in vivo* growth in 11/12 isolates tested as the clinical exposure unlikely to meet its pharmacodynamic targets at such MICs. One isolate, AB 319 with an MIC of 8 mg/L, exhibited bactericidal activity over the 72-h experiment, which is not unexpected as the clinical dose of cefiderocol may achieve its pharmacodynamic target because the $fT > MIC$ of 8 mg/L for the murine HSR was 80%.[21] Combination of cefiderocol with ceftazidime/avibactam or sulbactam (with ampicillin) resulted in marked bactericidal activity across all 12 test isolates. The activity of cefiderocol with ceftazidime/avibactam is expected as previous *in vitro* assays have determined the MICs of cefiderocol with avibactam reduced the MICs including in cefiderocol-non-susceptible *A. baumannii*.[10,13,14] Similar to the *in vitro* data, *in vivo* efficacy was seen in isolates with acquired serine β-lactamases that may be inhibited by avibactam (i.e. VEB and PER).[13,14] Decreases in MIC have been observed with avibactam against isolates that only harboured intrinsic oxacillinases and cephalosporinases, these *in vivo* data suggest that inhibition of cephalosporinases by avibactam may also enhance the activity of cefiderocol.[13,14] Cefiderocol in combination with sulbactam produced similarly impressive *in vivo* activity in the present study. Indeed, the present study used a murine HSR that mimicked the free plasma profile of high-dose, extended-infusion sulbactam (administered with ampicillin) that is advocated for in the IDSA guidance.[6] The mechanism behind the potent *in vivo* activity of the combination is unclear, however; in addition to sulbactam's antibacterial activity against *A. baumannii*, sulbactam can inhibit PER-type β-lactamases that may contribute to the efficacy for such isolates.[25] Sulbactam has been described to weakly inhibit ADCs intrinsic to *A. baumannii*, thus the high exposure of sulbactam may provide activity via ADC inhibition.[26] Some isolates assessed tested susceptible to sulbactam, thus activity of this agent alone at high doses and extended-infusion administration may contribute to the efficacy although most isolates tested were sulbactam-non-susceptible, which is consistent with <25% of carbapenem-resistant *A. baumannii* testing susceptible to sulbactam *in vitro*.[7] Meropenem combinations failed to consistently produce significant bacterial kill in the present study. Previous assessments of dual carbapenem therapy for *A. baumannii* postulated that one may act as a suicide inhibitor of carbapenemases produced by the organism.[27] This may have not led to clinically relevant bactericidal activity *in vivo* due to the diversity of enzymes produced as well as the other non-enzymatic resistance mechanisms likely present in this collection of highly resistant isolates.

Previous *in vivo* studies have demonstrated, although rare, post-exposure resistance to cefiderocol in *A. baumannii* has been observed.[10] Similar to our previous data, development of resistance was seen in both AB 237 and AB 230 with the latter occurring more frequently, showing consistency across the model.[10] Frequency of resistance for both isolates has been previously determined and was $4 \times 10{-}^{7}$ and $1 \times 10{-}^{6}$ for AB 230 and 237, respectively.[10] The present study added to these findings as for both isolates combination therapy with each test agent increased the *in vivo* activity and prevented the emergence of resistance in cefiderocol-susceptible isolates with MICs of 2 mg/L. For AB 147, cefiderocol with ceftazidime/avibactam and sulbactam (with ampicillin) also prevented resistance emergence on therapy. Conversely, the combination with meropenem observed

(a)

		Cefiderocol + ceftazidime/avibactam																					
		Stacked disc diffusion diameter																					
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
BMD Modal MIC	>32																							
	32																							
	16																							
	8																							
	4											3	3											
	2						1			3														
	1											2	1	2	2	4	1							
	0.5																1	2						
	0.25																1	4		1				
	0.125													1	1	1								
	0.06																1		2					

(b)

		Cefiderocol + sulbactam (with ampicillin)																					
		Stacked disc diffusion diameter																					
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
BMD Modal MIC	>32																							
	32																							
	16																							
	8									1	1	2	7	1										
	4								1	2	2	1	3	2	1									
	2												3	3		1								
	1												2	1				2		1				
	0.5																							
	0.25																							
	0.125																							
	0.06																							

(c)

		Cefiderocol + meropenem																						
		Stacked disc diffusion diameter																						
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
BMD Modal MIC	>32	7		1	1		2	1																
	32	3																						
	16	6		3	1		1	4	1															
	8																							
	4	1		2																				
	2																							
	1											1	1			1								
	0.5																							
	0.25																							
	0.125																							
	0.06																							

Figure 3. Distribution of the zone diameter of disc stacking of (a) cefiderocol + ceftazidime/avibactam, (b) cefiderocol + ampicillin/sulbactam and (c) cefiderocol + meropenem compared to the modal broth microdilution MIC of each respective combination. All disc diffusion zones are in at least three replicates per isolate. The red line corresponds to cefiderocol susceptibility per CLSI (BMD ≤ 4 mg/L, DD ≥ 15 mm). The blue line signifies cefiderocol intermediate per CLSI (BMD = 8 mg/L, DD = 11–14 mm). Green boxes indicate that >1-log10 reduction in cfu/thigh was observed in all isolates in each category. An orange box indicates at least one isolate in the category did not demonstrate >1-$\log_{10}$ reduction in cfu/thigh or was no better than cefiderocol alone. BMD = broth microdilution, DD = disc diffusion.

one thigh that had elevated MICs to cefiderocol post-exposure. This finding is consistent with the pharmacodynamic profile as AB 147 was the only cefiderocol-susceptible isolate where the cefiderocol and meropenem combination therapy resulted in similar reductions in $\log_{10}$ cfu/thigh compared with cefiderocol alone. The use of human-simulated exposure and a longer 72 h period allows for a translational assessment of not only *in vivo* efficacy, but also treatment emergent resistance, and may be a useful strategy to evaluate more target MDR organisms with other antibacterial combinations. These data provide foundational *in vivo* and microbiologic data to design rational combinations for clinical validation; however, practical *in vitro* assessments are needed to identify clinical isolates that are likely to respond to such combinations.

Various methods have been advocated to assess *in vitro* activity including checkerboard, time-kill, crossing of gradient

diffusion strips and disc diffusion studies.[28] An advantage of the gradient diffusion strips and disc diffusion-based methods is such procedures use materials commonly stocked in clinical microbiology laboratories. In the present study, we assessed *in vitro* activity using stacked discs as these materials are readily available for cefiderocol and all combination agents. Similar to previous data, the broth microdilution and disc stacking of ceftazidime/avibactam with cefiderocol resulted in the most prominent decrease in MIC and increase in zone of inhibition.[10,13] Similar findings were noted with ampicillin/sulbactam where, for both combinations, all zones of inhibition were returned to at least intermediate. This translated to *in vivo* activity using the human-simulated exposures for all test isolates. Indeed, the influence of both test agents must be considered as the treatments were administered in the clinically available formulations, and thus the return of MICs and zones of inhibition to the sulbactam intermediate zone in the presence of cefiderocol probably contributed to the *in vivo* efficacy. A practical approach for clinical isolates may be the achievement of at least a zone of inhibition that is interpreted as intermediate with stacked discs, which may indicate an isolate that would benefit from combination therapy. Similar to the *in vivo* data, the *in vitro* activity of cefiderocol with meropenem was variable. The lack of *in vitro* and *in vivo* correlation may discourage the viability of this combination as, despite the fact most isolates respond *in vivo*, we were unable to find an *in vitro* correlation to guide therapy. While data derived from the disc stacking method is encouraging, no standardized methods for disc stacking are endorsed by CLSI or EUCAST. Assessment of disc stacking in a larger cohort of clinical *A. baumannii* should be evaluated to better understand the *in vitro* response to the studied combinations as well as the effect of these combinations on clinical outcomes.

An advantage of the present analysis was the use of clinical, highly resistant isolates that may be encountered and leave clinicians no viable treatment options. Although a limitation may be that only 15 isolates were assessed, these isolates had various genotypic and phenotypic profiles, thus these findings are likely generalizable to larger cohorts of MDR *A. baumannii*. Additionally, we did not assess isolates with cefiderocol MICs ≤1 mg/L (cefiderocol MIC_{90} in CRAB)[9] because previous studies failed to find increases in potency when avibactam was added to cefiderocol for such isolates,[12] and previous *in vivo* studies have found cefiderocol monotherapy highly active.[10,21] Therefore, combination therapy may not provide an added benefit for highly susceptible isolates.

The present study is not without limitations. First, the discs used for combination *in vitro* testing contained fixed concentrations and ratios of each agent that may not have reflected the clinical exposures of the agents produced by the clinical doses in humans or the HSRs in mice. With that said, the *in vitro* findings provide an assessment of biologic plausibility that may be useful to identify isolates more likely to respond to each combination therapy *in vivo*. Indeed, the fixed concentration of antibiotics may under call synergy (as seen in the meropenem based experiments); this is preferable from a safety perspective as it reduces the risk of an *in vitro* test suggesting activity where it was not found *in vivo*. Second, not all monotherapies were assessed *in vivo*. Indeed, the five isolates tested against each monotherapy demonstrated that for isolates with elevated MICs to each agent (e.g. meropenem ≥8 mg/L, ceftazidime/avibactam >16 mg/L or sulbactam ≥8 mg/L) the combination with cefiderocol produced significantly better *in vivo* activity. The remaining cefiderocol-NS isolates assessed all had MICs within this range for each combination agent thus represent challenging clinical isolates. Combination of cefiderocol with ceftazidime/avibactam and sulbactam produced significant *in vivo* activity (e.g. > 1-log_{10} kill from baseline) against all isolates tested, suggesting these may represent rational combination therapy for highly resistant *A. baumannii*. These foundational *in vivo* data using human-simulated exposures require clinical validation to assess the effect of cefiderocol plus ceftazidime/avibactam or ampicillin/sulbactam on clinical outcomes for patients infected with *A. baumannii*.

In conclusion, human-simulated exposures of cefiderocol in combination with ceftazidime/avibactam or sulbactam (with ampicillin) resulted in potent *in vivo* activity against 15 carbapenem-non-susceptible *A. baumannii* including cefiderocol-non-susceptible isolates. Against three cefiderocol-high-end-susceptible isolates, these combinations also prevented the emergence of resistance *in vivo*. Cefiderocol in combination with meropenem resulted in more variable activity. Combination disc diffusion through disc stacking may be a feasible method to assess isolates that may respond to combination therapy. Disc stacking of cefiderocol and ceftazidime/avibactam or ampicillin/sulbactam, which results in a zone of inhibition that is indicative of the intermediate breakpoint, may indicate the isolate will respond *in vivo* at the studied clinical doses. Our findings are hypothesis generating, however, clinical validations of cefiderocol plus ceftazidime/avibactam or ampicillin/sulbactam therapy for *A. baumannii* and the impact on clinical outcomes are warranted.

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Supplementary data

Figures S1 and S2 and Tables S1 to S4 are available as Supplementary data at *JAC* online.

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