Assessment of sustained efficacy and resistance emergence under human-simulated exposure of cefiderocol against *Acinetobacter baumannii* using *in vitro* chemostat and *in vivo* murine infection models

Christian M. Gill1, Kamelia Abdelraouf2, Merime Oota2, Rio Nakamura2, Miho Kuroiwa3, Yoshino Ishioka3, Miki Takemura4, Yoshinori Yamano5 and David P. Nicolau1,6*

1Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, CT, USA; 2Shionogi TechnoAdvance Research & Co. Ltd, Osaka, Japan; 3Laboratory for Innovative Therapy Research, Shionogi & Co. Ltd, Osaka, Japan; 4Laboratory for Drug Discovery and Disease Research, Shionogi & Co. Ltd, Osaka, Japan; 5Research Planning Department, Shionogi & Co. Ltd, Osaka, Japan; 6Division of Infectious Diseases, Hartford Hospital, Hartford, CT, USA

*Corresponding author. E-mail: david.nicolau@hhchealth.org

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**Objectives:** This study evaluated the sustained kill and potential for resistance development of *A. baumannii* exposed to human-simulated exposure of cefiderocol over 72 h in *in vitro* and *in vivo* infection models.

**Methods:** Seven *A. baumannii* isolates with cefiderocol MICs of 0.12–2 mg/L were tested. The sustained bactericidal activity compared with the initial inoculum and the resistance appearance over 72 h treatment were evaluated in both an *in vitro* chemostat and an *in vivo* murine thigh infection model under the human-simulated exposure of cefiderocol (2 g every 8 h as 3 h infusion).

**Results:** In the *in vitro* model, regrowth was observed against all seven tested isolates and resistance emergence (>2 dilution MIC increase) was observed in five test isolates. Conversely, sustained killing over 72 h and no resistance emergence were observed in six of seven tested isolates *in vivo*. The mechanism of one resistant isolate that appeared only in the *in vitro* chemostat studies was a mutation in the tonB-exbB-exbD region, which contributes to the energy transduction on the iron transporters. The resistance acquisition mechanisms of other isolates have not been identified.

**Conclusions:** The discrepancy in the sustained efficacy and resistance emergence between *in vitro* and *in vivo* models was observed for *A. baumannii*. Although the resistance mechanisms *in vitro* have not been fully identified, sustained efficacy without resistance emergence was observed *in vivo* for six of seven isolates. These studies reveal the *in vivo* bactericidal activity and the low potential for development of resistance among *A. baumannii* evaluated under human-simulated exposures.

**Introduction**

Carbapenem-resistant *Acinetobacter baumannii* is considered a critical priority for antibiotic research and development by WHO due to high mortality, limited treatments and healthcare burden, among others.3 Bacteraemia and hospital/ventilator-associated pneumonia are two of the most common infections associated with *A. baumannii* and have been associated with mortality ranging from 37%–55%.2-4 Prior antibiotic use and hospitalizations are associated with *A. baumannii* infections and ineffective antimicrobial therapy has been associated with mortality in *A. baumannii* bacteremia.3,5

Indeed, carbapenem-resistant *A. baumannii* leaves clinicians with limited treatments due to high-level resistance to numerous antibacterials (i.e. β-lactams, aminoglycosides, and fluoroquinolones, among others).6,7 Cefiderocol remains active against carbapenem-resistant *A. baumannii* due to the utilization of active iron transporters to penetrate through the outer membrane and high stability to both serine- and metallo-type β-lactamases.8 Cefiderocol has shown potent antibacterial
activity in consecutive multinational surveillance studies (SIDERO-WT studies) against clinical isolates of A. baumannii in North America and Europe with MIC$_{90}$ of 1 to 4 mg/L, and inhibited the growth of 91.0% to 96.9% of the isolates at ≤4 mg/L. The acquisition of β-lactamases such as bla$_{OXY}$ has been reported to contribute to elevated cefiderocol MICs in clinical isolates of A. baumannii, although β-lactamase inhibitors such as avibactam have been shown to reduce cefiderocol MICs in such isolates.

Cefiderocol exhibited efficacy against Gram-negative bacteria including A. baumannii in multiple clinical studies including APEKS-NP and CREDIBLE-CR. In the APEKS-NP study, cefiderocol was non-inferior to high-dose, extended-infusion meropenem in terms of all-cause mortality on Day 14 in patients with Gram-negative nosocomial pneumonia, with similar tolerability. In the CREDIBLE-CR study, cefiderocol had similar clinical and microbiological efficacy to best available therapy (BAT) in the heterogeneous patient population with infections caused by carbapenem-resistant Gram-negative bacteria, although there was numerically higher all-cause mortality among cefiderocol treated patients with infections caused by Acinetobacter sp. In these studies, the frequency of ≥4-fold MIC increase during the treatment in cefiderocol arm was similar to those of BAT and meropenem arms although the magnitude of increase to cefiderocol was smaller. Although actual cefiderocol MIC values remained ≤4 mg/L for many of the isolates which showed ≥4-fold MIC increase, careful monitoring for the appearance of cefiderocol resistance will be important.

We previously reported the discrepancy of resistance emergence of Stenotrophomonas maltophilia in in vitro chemostat models and in vivo murine thigh infection models where resistant mutants from the chemostat model were likely non-viable in the in vivo environment. Thus, the present study sought to compare the potential of cefiderocol resistance development of A. baumannii in an in vitro chemostat model and an in vivo murine thigh infection model, both utilizing human-simulated regimen (HSR) exposures over an extended treatment period of 72 h.

**Materials and methods**

**Ethics**

The present study was approved by the Institutional Animal Care and Use Committee of Hartford Hospital (Assurance #A3185-01). All animal experiments were conducted in concordance with the standards set by the National Research Council of the National Academy of Sciences.

**Antimicrobial test agents**

Cefiderocol 500 mg vials (Shionogi & Co. Ltd, Osaka, Japan, lot: 12M01) were used for all in vitro and in vivo experiments. Cefiderocol was reconstituted with normal saline (NS) prior to being further diluted to the desired final dosing concentration in NS to use in in vivo studies. All doses were delivered as 0.2 mL injections subcutaneously.

**Isolates**

Nine clinical A. baumannii isolates were used. Seven of nine clinical isolates (AB230, AB231, AB232, AB233, AB235, AB236 and AB237) were used for both in vitro and in vivo studies. The two remaining clinical isolates (AB84 and AB87) were used in previously published in vivo experiments using the same model, and thus used for the in vivo studies as internal controls to validate the stability of the model and the consistency of the in vivo response to the cefiderocol HSR across studies.

The cefiderocol MICs for isolates used for both in vitro and in vivo studies were determined to be 0.12–2 mg/L by the broth microdilution (BMD) method using iron-depleted CAMHB (ID-CAMHB), which was prepared based on CAMHB (Becton-Dickinson, Sparks, MD, USA), as recommended by CLSI (Table 1).

In addition, the β-lactamase gene profile was also determined by WGS or PCR analysis (Table S1, available as Supplementary data at JAC-AMR Online) prior to inclusion in the study (Table 1). Isolates were randomly selected based on the cefiderocol MICs to have a larger population of the isolates with MIC of 1 or 2 mg/L, which is near the susceptibility breakpoint, to evaluate the risk of resistance emergence as well as having a relevant background of genotypically identified β-lactamases.

**In vitro chemostat studies**

Seven clinical isolates were used for the in vitro chemostat models to evaluate the bacterial killing and the emergence of resistance under the human pharmacokinetic (PK) exposure of cefiderocol for 72 h, as described previously. Briefly, an exponentially growing bacterial suspension of 5.00 to 5.78 log$_{10}$ CFU/mL was incubated at 37°C for 72 h in ID-CAMHB under conditions recreating the free cefiderocol concentration-time curves in plasma in healthy subjects (for AB230 and AB231) or patients, which was determined from the population PK studies in the patients from Phase II/III studies (for the remaining five isolates). The bacterial counts were determined at 4 h and every 8 h until 72 h by the incubation of 10-fold serially diluted bacterial suspension on drug-

| Isolate ID | MLST (PubMLST Oxford) | cefiderocol MIC (mg/L) | meropenem MIC (mg/L) | β-lactamase |
|------------|-----------------------|------------------------|---------------------|-------------|
| AB230$^a$  | 281                   | 2                      | 32                  | ADC-33, OXA-82 |
| AB231$^a$  | 281                   | 1                      | 32                  | ADC-33, OXA-23, OXA-82 |
| AB232$^a$  | 944                   | 0.125                  | >64                 | ADC-152, CARB-16, CTX-M-115, OXA-72, OXA-90 |
| AB233$^{a,b}$ | N/A$^e$              | 0.25                   | >64                 | ADC, OXA-24-like |
| AB235$^a$  | 281                   | 2                      | 64                  | ADC-33, OXA-23, OXA-82 |
| AB236$^a$  | 1418                  | 1                      | 32                  | ADC-52, CARB-16, ADC-199, OXA-23, OXA-91 |
| AB237$^{a,b}$ | N/A$^e$              | 2                      | 16                  | ADC, OXA-58-like |
| AB87$^d$   | ND                    | 4                      | ND                  | ADC-25, OXA-23, OXA-66, PER-1 |
| AB84$^d$   | 1289                  | 16                     | 32                  | ND |

N/A, not applicable; ND, not determined.
$^a$Evaluated in vitro and in vivo.
$^b$β-lactamase profile was determined by PCR.
$^c$Not identified.
$^d$Internal control isolates previously evaluated in the in vivo model to assess stability of the model.

Table 1. Clinical isolates included the in vitro and in vivo models
free tryptic soy agar (Becton-Dickinson). The cefiderocol MIC was determined by selecting five colonies that were randomly picked up from 72 h treatment cultures to make a bacterial suspension, which was then assessed with BMD methods using ID-CAMHB. Against the isolates with reduced susceptibility to cefiderocol, the cefiderocol MIC in the presence of avibactam (final concentration, 4 mg/L) was determined, since previous reports found avibactam augmented cefiderocol potency in vitro against some A. baumannii isolates with elevated cefiderocol MICs.10

Frequency of resistance
The bacterial suspensions of these strains prior to experimental antimicrobial exposure were used to evaluate the potential of resistance emergence observed in the chemostat studies, which showed ≥32-fold increase of MIC. A concentration of 10^2 or 10^3 cfu/mL was prepared by dilution of the culture grown overnight in ID-CAMHB and spread on Mueller–Hinton agar (MHA) containing 10-fold MIC of cefiderocol, respectively. After incubation at 35°C for 48 h, the colonies that appeared on the plates then underwent cefiderocol susceptibility testing.

WGS analysis and analyses of MLST and β-lactamase
The isolates that showed an increase in cefiderocol MIC in vitro chemostat studies were analysed using WGS. Genomic DNA from each bacterial sample was extracted and WGS was performed using the Illumina MiSeq system (Illumina, San Diego, CA, USA) as described previously.10,13 The raw FASTQ reads were first trimmed to a quality score limit of 0.05 (Q13) with maximum two ambiguous nucleotides and assembled into contigs for each test sample using CLC Genomics Workbench version 11.0.1 (Qiagen, Hilden, Germany) as described previously.10,13 To investigate β-lactamase genes, all contig datasets of test samples were loaded to the Pipeline Pilot version 18.1.1.100.11 (PP) which consists of β-lactamase gene database, NCBI gene (https://www.ncbi.nlm.nih.gov/gene/) with some manual curation. The subtype of β-lactamase was identified by the amino acid sequence of each gene. The A. baumannii MLST profiles were determined by comparison of seven allele sequences, which were determined by blastn search for generated contigs using the PP, from the public Oxford database (https://pubmlst.org/bigsdb/?db=pubmlst_Abaumannii_oxford_seqdef/).

Long-read sequencing and analyses
For AB231 and its resistant mutant, WGS was performed using both Illumina MiSeq short-read sequencing and Oxford Nanopore MinION (Oxford, UK) long-read sequencing technologies. The long-read sequencing and de novo assembly were performed at Gen李白 (Yokohama, Japan). MiSeq and Nanopore reads were assembled using Unicycles.21 The assembled contigs were error corrected using Nanopolish (available from https://github.com/jts/nanopolish). The protein-coding genes along with their functional annotation of the long contigs were predicted and specific mutations in AB231 derivative were detected using Genedata Selector 5.2.3 (Genedata, Switzerland). Detected mutations were confirmed by Sanger sequencing using BigDye Terminator v3.1.

mRNA expression analysis
The mRNA expressions were determined by real time RT-PCR. The RNA of A. baumannii in the exponential growth phase in ID-CAMHB was extracted with RNeasy mini kit (Qiagen). Real time RT-PCR was conducted with One Step PrimeScript RT-PCR Kit (Takara-bio, Shiga, Japan) and Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific) according to the manufacturer’s instruction. The mRNA expression of tonB, exbB and exbD genes were tested using strain AB231 and its derivatives, and those of blaDADC, blaOXA-23 and blaOXA-82 were tested using strains AB230 and AB235 and their derivatives. Comparative Ct method was used to determine the relative expression level of target genes using recA as a reference gene.

Animals
Specific-pathogen-free, female, CD-1 mice (20–22 g) were obtained from Charles River Laboratories Inc. (Raleigh, NC, USA). All animals acclimatized for 48 h prior to study procedures housed in groups of six animals per HEPA-filtered cage (Innovive, San Diego, CA, USA) at controlled room temperature. Cages were used with nourishment and enrichment as previously described.13 Monitoring for morbidity was conducted as previously described and animals were euthanized if found moribund and tissues were harvested.13

Neutropenic murine thigh infection model
Prior to all in vivo efficacy studies, animals were prepared as follows: neutropenia was induced using intraperitoneal (IP) cyclophosphamide 150 mg/kg and 100 mg/kg administered on Days −4 and −1, respectively. On study Day −3, 5 mg/kg of uranil nitrile was administered IP to aid in developing the cefiderocol HSR.22,23 An inoculum of −1 × 10^7 cfu/mL was needed as previously described to establish infection with this bacterium in the thigh model.15,16 The cefiderocol HSR was administered as previously described in the neutropenic murine thigh infection model to produce plasma exposure in the mice similar to that of cefiderocol 2 g IV every 8 h over 3 h in humans.14,15,22,23

In vivo efficacy studies
One group of three mice per isolate was sacrificed via CO2 asphyxiation and cervical dislocation at 0 h (2 h post-inoculation) and both thighs were harvested aseptically to enumerate baseline bacterial burden. Groups of three mice received either sham control (NS) or cefiderocol HSR subcutaneously for 24, 48 or 72 h. At each designated timepoint (24, 48 and 72 h), control and cefiderocol HSR groups were euthanized as described above and thighs were aseptically harvested. Bacterial enumeration was conducted on each thigh at each timepoint as previously described.13 Animals that were sacrificed due to morbidity or succumbed to infection were assessed at the closest following timepoint and thus some timepoints did not have any animals survive to be assessed. Efficacy was assessed using the change in log10 cfu/thigh for each treatment by subtracting the log10 cfu/thigh at each assessment point (24 h, 48 h or 72 h) from the 0 h control. Log10 change in cfu/thigh was reported as mean ± SD for each treatment per isolate. Achievement of 1 log10 bacterial kill at 24 h, 48 h and 72 h relative to the 0 h controls was assessed as a surrogate endpoint for prediction of clinical efficacy.24

Resistance determinant study in the in vivo model
To evaluate resistance development during cefiderocol treatment, post-exposure MICs were determined for isolates recovered during the in vivo efficacy studies from the infected murine thighs as previously described.13 Post-exposure MICs were determined by BMD per CLSI standards using made-to-order ID-CAMHB produced by Thermo Fisher (YT346A-5, Oakwood Village, OH, USA, lot: M20261).16,17 Quality control was conducted with CLSI guidance using Pseudomonas aeruginosa ATCC 27853 and MIC endpoints were determined as outlined in the CLSI guidelines where trailing was not interpreted as the endpoint.16 The post-exposure BMD MICs were considered to have resistance development if the cefiderocol-treated isolates had a greater than two dilution increase in the MIC compared with control (saline)-treated isolates. Post-exposure specimens with recoverable colonies were frozen at −80°C in skim milk.
In vitro chemostat studies

Cefiderocol showed greater than 2 log_{10} kill against all seven test strains with MICs of 0.12 to 2 mg/L within 8 h (>3 log_{10} reduction for four strains and 2 to 3 log_{10} reduction for three strains) under the human exposure in the chemostat models, but regrowth was observed for all cases during the 72 h treatment (Figure 1). Reduced susceptibility was observed in five of the seven isolates assessed. Resistant colonies were not obtained for isolates AB233 and AB237. For the remaining five isolates (AB230, AB231, AB232, AB235 and AB236), 5/5, 5/5, 2/5, 5/5 and 5/5 isolates showed >32-fold increase in cefiderocol MIC, and the MIC of these five isolates after 72 h treatment ranged from 8 to >128 mg/L. As for AB230 and AB235, which showed regrowth within 24 h treatment, the appearance of the resistant isolates was confirmed in the bacterial suspension at 24 h treatment, and the MIC of the resistant isolates from 24 h and 72 h treatment was the same.

The resistance phenotype observed was reversed by the addition of avibactam to the MIC testing of three isolates. In the case of AB230 and AB235, which showed regrowth within 24 h treatment, the MIC of the resistant isolates was confirmed in the bacterial suspension at 24 h treatment, and the MIC of the resistant isolates from 24 h and 72 h treatment was the same. The resistance phenotype observed was reversed by the addition of avibactam to the MIC testing of three isolates. In the case of AB230 and AB235, which showed regrowth within 24 h treatment, the appearance of the resistant isolates was confirmed in the bacterial suspension at 24 h treatment, and the MIC of the resistant isolates from 24 h and 72 h treatment was the same.

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The frequency of the colonies that appeared on the agar medium at 24 h treatment among these seven test strains. As for AB231, no colonies appeared on the agar medium in the frequency of resistance study, but resistant mutants appeared in the chemostat models. On the other hand, for AB233 and AB237, colonies appeared on the agar medium, but resistant mutants were not obtained in the chemostat models. When the mutants with decreased cefiderocol susceptibility were phenotypically assessed with and without the addition of avibactam, similar MICs were seen regardless of which study they were derived from (e.g. frequency of resistance versus chemostat study).

WGS analysis

Assessing the AB231 resistant mutants, no mutation was observed by the short-read analysis, but the insertion of IS5 family transposase ISAba1 (1189 bp with 99% identity with ISAba1) at the stop codon of the tonB gene was observed by long-read analysis in all five resistant mutants derived from this strain in the in vitro chemostat studies. As a result, this mutation caused the addition of eight amino acid residues after the C-terminus amino acid residue of the original TonB protein and 20-fold decrease of the mRNA expression of exbB and exbD genes, which are located downstream the tonB gene as tonB-exbB-exbD operon. In addition, the expression level of multiple β-lactamase genes was observed using AB230, which had ADC-33 and OXA-82, and AB235, which had ADC-33, OXA-23 and OXA-82, and their corresponding cefiderocol-resistant mutants obtained in chemostat models. For both isolates, neither gene mutations nor different expression level were observed as determined by RT-PCR.

In vivo efficacy study

Two A. baumannii isolates previously evaluated in the model (AB87 and AB84) produced similar reduction and increase in bacterial burden to previously published data, respectively. Figure 2 displays the change in bacterial burden including averaged results for AB87 and AB84 internal controls.

Mean bacterial burden at 0 h for the other seven A. baumannii isolates tested in the model was 5.63 ± 0.31 log_{10} cfu/thigh. In all seven A. baumannii isolates tested, there was an increase in bacterial burden in control animals at each timepoint in the absence of mortality (Figure 2). The mean change in bacterial burden for cefiderocol treated animals ranged from −2.78 to +0.49 log_{10} cfu/thigh, −3.07 to +1.20 log_{10} cfu/thigh and −3.26 to +0.43 log_{10} cfu/thigh at 24, 48 and 72 h, respectively. Cefiderocol HSR produced net bacterial stasis in the initial 24 h for isolates AB230 and AB231; however, AB231 displayed bacterial reduction at 48 and 72 h compared with continued bacteriostasis with AB230. Mean bacterial reductions were noted at 24 h and sustained over 48 and 72 h in the remaining five A. baumannii isolates. Overall, 4/7, 6/7 and 5/7 isolates achieved 1 log_{10} kill at 24, 48 and 72 h, respectively (Figure 2). Additionally, 2 log_{10} kill was achieved in three of seven and four of seven isolates at 48 and 72 h, respectively.

Resistance determination studies of in vivo samples

For the seven A. baumannii isolates tested, 57 samples had sufficient growth post-exposure to conduct MIC testing. For isolates AB232 and AB237, each had a single specimen where the post-exposure BMD MIC was >2-fold dilution higher than corresponding controls at 72 and 48 h, respectively. Disc diffusion testing for both AB232 and AB237 recovered from cefiderocol-treatment groups with elevated BMD MICs resulted in zone diameters similar to the bacteria recovered from the control mice as well as a fresh culture from the frozen stock without prior cefiderocol exposure. Thus, the disc diffusion confirmation studies on AB232 and AB237 are suggestive of variability in the interpretation of the BMD testing results not overt development of resistance. AB230 had two, three and one sample at 24, 48 and 72 h with elevated BMD MICs, respectively. Disc diffusion from a single sample at 48 h and 72 h resulted in a decreased zone of inhibition consistent with the phenotypic change observed by BMD. All other post-exposure AB230 samples had disc diffusion zones similar to those of controls and the frozen bacterial stock without cefiderocol exposure.

Discussion

In this study, the sustained bactericidal efficacy and resistance acquisition under human-simulated exposure were evaluated using an in vitro chemostat and an in vivo murine thigh infection.
Figure 1. Bactericidal activity against Acinetobacter under humanized exposures.
The results showed a discrepancy where in vivo cefiderocol HSR produced sustained efficacy and limited resistance development compared with regrowth and resistance as seen in vitro. These findings were similar to the observation with S. maltophilia.13 The present study reaffirmed the in vivo activity of cefiderocol HSR against six of the seven A. baumannii isolates tested. The greater than 1 log_{10} kill target associated with clinical outcomes in humans was achieved in four to six of the seven isolates tested depending on timepoint assessed, suggesting sustained microbiological activity over the 72 h model. This finding is consistent with pharmacokinetic/pharmacodynamic (PD) indices of fT_{>MIC} predictive of success in murine infection models, including A. baumannii.25 Of note, the magnitude of the fT_{>MIC} target for 1 log_{10} kill for A. baumannii was relatively higher than that of other tested species with 88% fT_{>MIC} compared with 64%–82% for Enterobacterales and P. aeruginosa.22,25 The cefiderocol exposure in humans receiving 2 g IV every 8 h over 3 h, like the murine regimen used in this study, would be predictive of efficacy for MICs up to the CLSI breakpoint of 4 mg/L (Table 3) as supported by the microbiological success observed for AB235, AB237 and AB87, which had cefiderocol MICs of 2, 2 and 4 mg/L, respectively.16–18 Indeed, inter-strain differences in the fT_{MIC} magnitude have been described and may explain the limited activity of cefiderocol against AB230 (MIC = 2 mg/L). Alternatively, resistance emergence may explain the limited efficacy against AB230.

Among the isolates tested, one sample each from AB232 and AB237 recovered post-cefiderocol exposure had BMD MICs that were increased 2-fold dilutions compared with isolates recovered from control treated animals; however, the magnitude of these phenotypic changes was unconfirmed by the disc diffusion methodology, although testing was conducted after freezing so expression derived mechanisms could not be ruled out. Conversely, AB230 was the only isolate noted to have increased MICs relative to controls confirmed using both microbiological methods. Indeed, this isolate resulted in suppression of growth in vivo in the neutropenic model over the entire 72 h study. This in vivo efficacy profile contrasts with the development of adaptive-based resistance observed with previous siderophore antibiotic conjugate candidates, where significant bacterial regrowth comparable in magnitude to the control counterparts was observed.26,27 Similarly, adaptive-based resistance with previous siderophore antibiotic conjugate candidates was seen

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**Table 2.** MIC and frequency of resistance for the test isolates derived from the in vitro model

| Isolate ID | Frequency of resistance | Cefiderocol MIC (mg/L) | Pre-treatment | Post-treatment |
|------------|-------------------------|------------------------|---------------|---------------|
| AB230      | 4.0 x 10^{-7}           |                        | 2             | 1             |
| AB231      | <9.1 x 10^{-8}          |                        | 1             | 0.25          |
| AB232      | 5.5 x 10^{-7}           | ≤0.06                  | 8             | 1             |
| AB233      | 1.1 x 10^{-6}           | NT                     | ≤0.06         | ≥8            |
| AB235      | 5.0 x 10^{-6}           | 2                      | >128          | 2             |
| AB236      | 3.0 x 10^{-6}           | 0.12                   | 64            | 0.5           |
| AB237      | 1.0 x 10^{-6}           | 2                      | NT            | NT            |

NT, not tested.

Avibactam tested at 4 mg/L.

Not done due to no resistance emergence.

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**Figure 2.** Change in bacterial density (mean ± SD) for untreated controls or mice receiving cefiderocol HSR against A. baumannii isolates including two internal controls (AB87 and AB84) and seven test isolates (AB230, AB231, AB232, AB233, AB235, AB236 and AB237). The absence of a result at a given timepoint represents no animals survived to the given timepoint in the group. Cefiderocol BMD MICs (mg/L) in parentheses were determined in iron-depleted media.
earlier in the experimental timeline at 24 h, which was not observed in the present study.26,27

The addition of avibactam reduced the cefiderocol MIC against the post-exposure resistant isolates from the chemostat model, including AB230, which was the only isolate that had sustained MIC increase after in vivo exposure. Indeed, the reductions in MICs varied by strain suggesting multiple mechanisms are contributing to elevated MICs. Although the increased production of any β-lactamases was not observed in the resistant mutants derived from at least two isolates (AB230 and AB235), the resistance mechanisms may be due to the induced production of β-lactamases in combination with decreased outer membrane permeability. The addition of avibactam is able to reduce the MIC by inhibition of β-lactamases that may free the cefiderocol to exert antimicrobial activity.19 Due to the multitude of mechanisms of resistance harboured by A. baumannii, combination therapy has been suggested.7 Despite this advocacy for multiple drug therapy; no consensus exists on optimal combination therapy or its impact on clinical outcomes and emergence of resistance.2,10 In vivo investigations using human-simulated exposures are needed to identify rational cefiderocol-based combination therapies to evaluate microbiological efficacy and prevention of resistance in A. baumannii.

Finally, WGS analysis of the resistant mutants derived from AB231 found a mutation in the tonB-exbB-exbD region with ISaBa1 insertion at the C-terminus of TonB protein, and the reduced expression of exbB and exbD genes. This mutation was not found in any of 168 clinical isolates from A. baumannii which could cause the loss of the ability to acquire siderophore-iron complex due to the deficiency of energy transduction system to acquire iron via siderophore-iron receptor.28 The resistant mutants caused by the mutation in the tonB-exbB-exbD region did not manifest in the in vivo model, an observation that may be due to decreased fitness associated with reduced iron acquisition. The mutation in this region was also observed for S. maltophilia, and this could be related with the discrepancy of the resistance emergence between in vitro and in vivo studies.13,28 The mechanisms that conferred resistance to the other isolates were not identified by WGS. Similar discordant findings between in vitro and in vivo pre-clinical models have been due to inefficient clearance of β-lactamases in the in vitro system causing decreased microbiological killing of β-lactam antimicrobials.26,29 This was also seen in experiments comparing the in vitro and in vivo efficacy of ceftazidime/avibactam where regrowth was seen in vitro but not in vivo, suggesting the physiological clearance of β-lactamases in the in vivo model may better describe what is seen clinically compared with the in vitro systems.26,29 Considering the variation in cefiderocol MIC reductions in the presence of avibactam, multiple mechanisms are likely involved.

A. baumannii represents a challenging clinical pathogen as the evaluation of patient outcomes is confounded by many factors.30 Notably, patients infected with A. baumannii typically have multiple acute and chronic conditions that may be associated with morbidity and mortality making outcome determination challenging.30

Indeed, the CREDIBLE-CR study evaluated clinical outcomes of patients with carbapenem-resistant infections between cefiderocol-treated patients and best available therapy.12 There was a mortality imbalance noted in patients with A. baumannii infections although clinical and microbiological outcomes were similar between groups. The present translational PK/PD murine model using clinically achievable cefiderocol exposures supports the microbiological efficacy of the agent against challenging A. baumannii clinical isolates. Translational data including combination therapy may better guide future clinical investigations for optimal therapy against difficult-to-treat A. baumannii infections.

In conclusion, a cefiderocol HSR mimicking the clinical dose of 2 g IV every 8 h over 3 h in humans displayed notable and sustained bacterial kill over 72 h in the neutropenic murine thigh infection model against A. baumannii isolates and the development of resistance was rare. The discrepancy of the efficacy and resistance emergence between in vitro and in vivo was observed, and the resistance acquisition observed in the in vitro chemostat model was partly due to the reduced ability to acquire iron by the mutation in tonB-exbB-exbD region, which developed in the highly enriched broth culture media. These data further support the in vivo activity of cefiderocol against A. baumannii.

Table 3. Comparative cefiderocol PK profiles in the in vitro chemostat and the in vivo murine model

| Drug | Model | % fT>MIC at MIC (mg/L) of: | 2 | 4 | 8 | 16 | 32 | 64 |
|------|-------|---------------------------|---|---|---|----|----|----|
| Cefiderocol 2 g IV q8h, 3 h infusion | Human, infected patients Phase II/IIIa | 100 | 100 | 100 | 100 | 38 | 0 |
| | In vitro chemostatb | 100 | 100 | 100 | 96 | 39 | 0 |
| | Human, healthy volunteers | 100 | 99 | 76 | 48 | 11 | 0 |
| | Mouse | 100 | 96 | 80 | 45 | 9 | 0 |

The PK of the in vitro chemostat model mimicked the free-plasma profile of infected patients from the Phase III trials.18 Murine free-plasma PK mimicked the human free-plasma cefiderocol PK from healthy volunteers.14,15,22,24

| Calculated from the steady-state PK for the pneumonia patient with creatinine clearance of 70 mL/min, body weight of 70 kg and albumin concentration of 3.0 g/dL. |

| Calculated from the non-steady-state PK for the pneumonia patient with creatinine clearance of 70 mL/min, body weight of 70 kg and albumin concentration of 3.0 g/dL. |

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Transparency declarations

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Supplementary data
Table S1 is available as Supplementary data at JAC-AMR Online.

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