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In vivo translational assessment of the GES genotype on the killing profile of ceftazidime, ceftazidime/avibactam and meropenem against *Pseudomonas aeruginosa*

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Objectives: To evaluate the *in vivo* killing profile of human-simulated exposures of ceftazidime, ceftazidime/avibactam and meropenem against GES-harbouring *Pseudomonas aeruginosa* in the murine thigh infection model.

Methods: Five *P. aeruginosa* isolates [three isogenic (GES-1, GES-5 and GES-15) and two clinical (GES-5 and GES-15)] were evaluated. MICs were determined using broth microdilution. Human-simulated regimens (HSRs) of ceftazidime 2 g IV q8h as a 2 h infusion, ceftazidime/avibactam 2.5 g IV q8h as a 2 h infusion and meropenem 2 g IV q8h as a 3 h infusion were administered. Change in bacterial burden relative to baseline was assessed.

Results: Modal MICs ranged from 8 to >64 mg/L for ceftazidime, from 1 to 16 mg/L for ceftazidime/avibactam and from 1 to >64 mg/L for meropenem. *In vivo*, for the isogenic strains, avibactam augmented ceftazidime activity against the GES-1- and GES-15-harbouring isolates. Both ceftazidime and ceftazidime/avibactam resulted in significant kill against the GES-5 isogenic isolate. The meropenem HSR produced >1 $\log_{10}$ kill against each isogenic isolate (MICs of 1–4 mg/L). Against the GES-5 clinical isolate, ceftazidime and ceftazidime/avibactam resulted in >1 $\log_{10}$ kill compared with bacterial growth with the meropenem HSR. In the clinical isolate harbouring GES-15, the elevated MICs of ceftazidime and ceftazidime/avibactam reduced the effectiveness of both compounds, while the observed reduction in meropenem MIC translated into *in vivo* efficacy of the HSR regimen, predictive of clinical efficacy.

Conclusions: In GES-harbouring *P. aeruginosa*, quantitative reductions in bacterial density observed with the translational murine model suggest that the phenotypic profile of ceftazidime, ceftazidime/avibactam and meropenem is predictive of clinical efficacy when using the evaluated dosing regimens.

Introduction

Antimicrobial resistance in bacterial pathogens had an estimated 4.95 million attributable deaths in 2019.[1] *Pseudomonas aeruginosa* remains one of the top six implicated organisms associated with greater than 250 000 deaths in the same year.[1] *P. aeruginosa* is associated with a high propensity for antimicrobial resistance, due to both intrinsic and acquired mechanisms. Intrinsically, *P. aeruginosa* is notorious for antimicrobial resistance, due to porin/efflux and β-lactamases (e.g. cephalosporinases).[2] *P. aeruginosa* has been described to acquire numerous other β-lactamases, including carbapenem-hydrolysing enzymes, such as MBLs (i.e. VIM, IMP, NDM) and serine carbapenemases (i.e. KPC), which are associated with resistance to our novel cephalosporin-β-lactamase inhibitor combinations, ceftolozane/tazobactam and ceftazidime/avibactam.[2,3] Another class of increasingly reported β-lactamases in *P. aeruginosa* are GES β-lactamases, which have been associated with MDR, including ceftolozane/tazobactam resistance.[3–5]

GES β-lactamases are a diverse class of serine β-lactamases, originally described as ESBLs (i.e. GES-1), due to hydrolysis of ceftazidime.[6] Over 27 subtypes have been described and a number of variants (i.e. GES-2, GES-5) have single amino acid substitutions that expand the hydrolytic activity to include carbapenems.[6,7] Indeed, differences in the *in vitro* catalytic testing methodologies and antibiotics tested make comparing different variants and the clinical implications of the different variants

challenging.[7] A number of recent studies have identified GES β-lactamases in carbapenem-resistant *P. aeruginosa* from around the globe.[3,5,8] In a cohort of patients with *P. aeruginosa* bacteraemia, infection with a GES-5-harbouring isolate was associated with higher 30 day mortality, highlighting the need to optimize detection and therapeutic options.[8] Novel diagnostics in clinical development are expanding their detection capabilities to include GES, which, integrated with *in vitro* MIC testing, may help to identify antimicrobials active against such isolates.[9] However, due to the limited detection capabilities, we currently lack clinical data to elucidate the best available therapies for *P. aeruginosa* with this emerging resistance determinant.

A recent report described a patient successfully treated with ceftazidime/avibactam plus colistin for a catheter-related bloodstream infection with an MDR *P. aeruginosa* carrying a GES-5 enzyme (carbapenemase).[10] During ceftazidime/avibactam therapy, a rectal surveillance swab remained positive for an MDR *P. aeruginosa* that harboured a GES-15 enzyme that displayed a reduced MIC of meropenem and an increased MIC of ceftazidime/avibactam compared with the original GES-5-harbouring isolate. The *in vivo* consequences of the differences between these enzyme subtypes is unknown. Herein, we used a previously validated murine infection model to assess the pharmacodynamic consequences of different GES variants in both clinical and isogenic *P. aeruginosa* using human-simulated regimens (HSRs) of ceftazidime, ceftazidime/avibactam and meropenem.

Materials and methods

Ethics

The present study was approved by the Institutional Animal Care and Use Committee of Hartford Hospital (A-HHC-2021-0309). All animal experiments were conducted in accordance with the standards set by the National Research Council of the National Academy of Sciences.

Antimicrobial test agents

Commercially available meropenem (Fresenius Kabi, CA, USA; Lot: 4A2OE14) and ceftazidime (Teligent Pharma, Inc., NJ, USA; Lot: FZG001) were used for all *in vivo* experiments. Analytical-grade avibactam (MedChem Express, NJ, USA; Lot: 23312) was used for both *in vitro* MIC and *in vivo* experiments. MIC trays were produced using analytical-grade ceftazidime (MedChem Express, NJ, USA; Lot: 64042) and meropenem (Sigma–Aldrich, WY, USA; Lot: LRAB7853).

Isolates

Five *P. aeruginosa* isolates, as previously reported,[10] were assessed in the current *in vivo* and *in vitro* experiments. Briefly, isolate PSA 2139 was a clinical isolate cultured from the blood of a patient catheter-related bloodstream infection and was determined to harbour a GES-5 carbapenemase (G170S variant of GES-1). Isolate PSA 2140 was derived from a rectal screen from the same patient while on treatment with ceftazidime/avibactam and was genotypically identical to PSA 2139; however, it harboured a GES-15 ESBL, which was likely a result of mutational changes (P162S) to the original GES-5. Neither clinical isolate displayed stably derepressed AmpC expression, as previously described.[10] PSA 2136, 2137 and 2138 were lab-derived isogenic PAO1 isolates producing cloned (pUCP24) GES-1 (ESBL), GES-5 (carbapenemase) and GES-15 (ESBL), respectively.[10]

Isolates were stored frozen at −80°C in skimmed milk and subcultured twice on trypticase soy agar with 5% sheep's blood. Isolates grew for 18–24 h at 37°C in ambient air to reach log phase prior to inoculation.

In vitro MIC testing

Broth microdilution MICs were determined in accordance with CLSI standards for at least five replicates using *P. aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* 700603 as quality controls; modal MIC values are reported for each isolate.[11,12]

Animals

Specific-pathogen-free, female, CD-1 mice (weight 20–22 g) were obtained from Charles River Laboratories, Inc. (Raleigh, NC, USA). All animals were acclimatized for the 48 h prior to all study procedures. Groups of six animals were housed in HEPA-filtered cages (Innovive, San Diego, CA, USA) at controlled room temperature. Nourishment and enrichment were provided, as previously described.[13]

Neutropenic murine thigh infection model

All animals were pretreated with cyclophosphamide [150 mg/kg intraperitoneally (IP) on day −4, 100 mg/kg IP on day −1] and uranyl nitrate (5 mg/kg IP on day −3), as previously described.[13,14] Animals were inoculated with a 0.1 mL injection of a bacterial suspension of $\sim 1 \times 10^7$ cfu/mL into one thigh per mouse.[13,14] Ceftazidime and ceftazidime/avibactam were administered using HSRs that resulted in plasma exposure observed with 2 g IV q8h as a 2 h infusion and 2.5 g IV q8h as a 2 h infusion, respectively.[14] All doses were administered as 0.2 mL injections subcutaneously with ceftazidime and avibactam co-administered at different concentrations across the dosing interval to produce exposures of each agent that mimic its exposure in human plasma[13] (ceftazidime: 4.5, 16, 13, 4.5, 3, 16, 13, 4.5, 3, 16, 13, 4.5, 3, 16, 13, 4.5, 3, 16, 13, 4.5 and 3 mg/kg at 0, 0.75, 2.25, 4, 6, 8.75, 10.25, 12, 14, 16.75, 18.25, 20 and 22 h; ceftazidime/avibactam: 7/0.875, 20/2.5, 7.5/1.25, 1/0.125, 13/1.625, 5/0.833, 13/1.625 and 5/0.833 mg/kg at 0, 0.75, 2.25, 4, 8.75, 10.25, 16.75 and 18.25 h). Meropenem was administered to simulate the human plasma profile of 2 g IV q8h as a 3 h infusion.[13] Meropenem was also administered as 0.2 mL subcutaneous injections[14] (meropenem: 65, 65, 45 and 45 mg/kg at 0, 1.25, 3.5 and 6 h, repeated every 8 h). Ceftazidime and ceftazidime/avibactam were evaluated, due to growing data for the *in vitro* susceptibility to ceftazidime/avibactam of GES-harbouring *P. aeruginosa*, as well as to evaluate if avibactam augments the activity of ceftazidime alone as suggested *in vitro*.[3] Meropenem was selected as it is a commonly used carbapenem and thus can provide insight into the *in vivo* efficacy of carbapenems against GES variants classified as ESBLs versus carbapenemases, particularly as GES-15 was noted to result in a lower meropenem MIC for the clinical case.[10]

In vivo efficacy studies

Each experimental group contained six mice per group, since optimal antimicrobial therapy typically produces 2–3 $\log_{10}$ kill and to tolerate a coefficient of variation of 40% in bacterial density, which would provide 80% probability that the mean is no more than one standard deviation from the true mean. Following post-inoculation randomization, groups of six mice per isolate were sacrificed at 2 h (0 h control group) via CO_2 asphyxiation and cervical dislocation, and the inoculated thigh was aseptically harvested to determine the baseline bacterial burden. The remaining groups of six mice per isolate received treatment starting 2 h after inoculation to allow bacteria to return to log-phase growth. Animals were randomized to receive saline control (24 h control group), ceftazidime HSR, ceftazidime/avibactam HSR or meropenem HSR dosed

subcutaneously for 24 h. After 24 h of dosing, the inoculated thigh was aseptically removed, homogenized in normal saline and cultured for bacterial enumeration, as previously described, via serial dilution.[13,14] Changes in $\log_{10}$ cfu/thigh are reported as mean ± SD and outliers were excluded if $\log_{10}$ cfu/thigh was outside of Tukey's hinges (first quartile − 1.5 × IQR and third quartile + 1.5 × IQR). The efficacy of treatment against the isogenic strains was benchmarked by the magnitude of bacterial kill compared with the expected kill associated with the produced $fT_{>MIC}$. The translation value of the humanized exposures against the clinical isolates was assessed using the model-derived surrogate endpoint, achievement of 1 $\log_{10}$ kill, predictive of clinical efficacy and serving as a more conservative target compared with alternative endpoints (i.e. bacteriostasis).[15] Changes in $\log_{10}$ cfu/thigh after treatment with ceftazidime, ceftazidime/avibactam and meropenem HSRs were assessed using one-way ANOVA followed by Tukey's post-hoc test if significant. A P value <0.05 signified statistical significance.

Results

In vitro potency and pharmacodynamic profiles of the test agents

For the three constructed isolates, avibactam augmented ceftazidime *in vitro* activity, with a ≥3 dilution reduction in modal MICs (Table 1). Regardless of the GES β-lactamase variant classification as an ESBL or carbapenemase, meropenem MICs ranged between 1 and 4 mg/L.

Considering the clinical isolates, the GES-5-harbouring isolate resulted in MICs 1 dilution higher for both ceftazidime and ceftazidime/avibactam compared with the isogenically inserted GES-5. Meropenem MICs were >64 mg/L for the clinical GES-5-harbouring isolate. The mutational changes in the GES-15 clinical isolate resulted in higher MICs of both ceftazidime and ceftazidime/avibactam (>64 and 16 mg/L, respectively), while meropenem displayed enhanced potency, with an MIC of 8 mg/L. Table 1 shows the known genotypic profiles for and modal MIC values of each isolate, as well as the resultant *in vivo* pharmacodynamic profile (i.e. plasma % $fT_{>MIC}$) for each treatment regimen.

In vivo efficacy study

In vivo, infection was established with a baseline bacterial burden of 5.86 ± 0.32 $\log_{10}$ cfu/thigh (PSA 2136, 6.15 ± 0.03 $\log_{10}$ cfu/thigh; PSA 2137, 6.09 ± 0.06 $\log_{10}$ cfu/thigh; PSA 2138, 6.03 ± 0.07 $\log_{10}$ cfu/thigh; PSA 2139, 5.57 ± 0.2 $\log_{10}$ cfu/thigh; PSA 2140, 5.40 ± 0.03 $\log_{10}$ cfu/thigh). All isolates displayed robust growth of >3 $\log_{10}$ change in cfu/thigh for untreated controls (range 3.45–4.26 change in $\log_{10}$ cfu/thigh).

Considering the constructed isolates, avibactam potentiated the *in vivo* activity of ceftazidime against the isolates harbouring GES-1 (−2.44 versus +3.15 change in $\log_{10}$ cfu/thigh) and GES-15 (−2.27 versus +1.09 change in $\log_{10}$ cfu/thigh) (Figure 1). Ceftazidime/avibactam kill and ceftazidime failure was consistent with the >70% and 0% $fT_{>MIC}$ produced by each HSR, respectively. Against the GES-5-harbouring isolate, ceftazidime/avibactam and ceftazidime produced a similar reduction in bacterial density (−2.56 versus −2.3 change in $\log_{10}$ cfu/thigh), which was expected, as at MICs of 1 and 8 mg/L, each HSR meets >70% $fT_{>MIC}$, which predicts efficacy for ceftazidime. Meropenem remained active *in vivo* against all three isolates, as anticipated, due to the pharmacodynamic profile provided by the high-dose, extended-infusion exposure in the context of the conserved phenotypic profile, MICs 1–4 mg/L, of the compound (Figure 1).

Considering the clinical GES-5-harbouring isolate (Figure 2), humanized exposures of both ceftazidime (MIC 16 mg/L) and ceftazidime/avibactam (MIC 2 mg/L) produced significant *in vivo* activity, consistent with their respective phenotypic profiles (−1.97 versus −2.52 change in $\log_{10}$ cfu/thigh). In contrast, bacterial growth of 3.46 $\log_{10}$ cfu/thigh was observed with the meropenem HSR (MIC >64 mg/L). Conversely, the mutational changes in the GES-15-harbouring isolate resulted in growth for the ceftazidime HSR (MIC >64 mg/L) and bacteriostasis for the ceftazidime/avibactam HSR (MIC 16 mg/L), with +3.93 ± 0.16 versus −0.35 ± 1.46 change in $\log_{10}$ cfu/thigh, respectively. The mutation in GES resulted in enhanced meropenem potency (MIC 8 mg/L) and *in vivo* activity (−2.04 ± 0.14 $\log_{10}$ cfu/thigh), as would be expected, due to the phenotypic profile.

Discussion

The present study used a validated translational murine infection model to evaluate the *in vivo* pharmacodynamics of ceftazidime, ceftazidime/avibactam and meropenem under human-simulated exposures against *P. aeruginosa* with GES β-lactamases. The differing hydrolytic spectrum of GES variants (i.e. ESBL versus carbapenemase) make it challenging to devise therapeutic strategies for isolates harbouring this diverse group of enzymes. The murine infection model with human-simulated exposures can provide necessary data to evaluate therapeutic implications of MDR isolates harbouring different β-lactamase variants, since antibiotic exposures used in the model are clinically achievable.[16]

Using the three constructed isolates, the present study found that GES genotypes corresponding to both ESBL and carbapenemase activity failed to elevate the meropenem MICs high enough to produce microbiological failure in the context of the wild-type *P. aeruginosa*. It must be noted, meropenem murine exposures mimicked those seen in humans receiving high-dose, extended-infusion meropenem (2 g IV q8h as a 3 h infusion), which would be predicted to produce bacterial kill up to an MIC of 8 mg/L, due to reaching the requisite 40% $fT_{>MIC}$ up to that MIC.[17] Conversely, when considering the clinical isolate that harboured GES-5 and background *oprD* and efflux, the meropenem therapy failed, as anticipated by the MIC of >64 mg/L, suggesting the sum of all these mechanisms may contribute to the phenotype and pharmacodynamic findings.

Although *in vitro* data suggest most GES-harbouring *P. aeruginosa* will have MICs indicative of high-level meropenem resistance (MIC_{90} >32 mg/L), a scenario where the compound would be expected to fail as observed with PSA 2139, the lower MIC for the GES-15 isolate allows an assessment of *in vivo* exposure response within the pharmacodynamic spectrum achievable in the clinical setting.[18] Interestingly, the mutational changes of GES-15 resulted in lower meropenem MICs, analogous with the previous findings of Haidar *et al.*,[19] where KPC-3 variants associated with ceftazidime/avibactam resistance in *K. pneumoniae* resulted in an 'ESBL phenotype', with significant decreases in the carbapenem MICs for variant-harbouring isolates. Our data suggest that the meropenem MIC (8 mg/L) for this

Table 1. Isogenic and clinical isolates included in the *in vivo* model

Isolate ID	Genotypic resistance determinants	Ceftazidime modal MIC (mg/L); murine $fT_{>MIC}$	Ceftazidime/avibactam modal MIC (mg/L)[a]; murine $fT_{>MIC}$[b]	Meropenem modal MIC (mg/L); murine $fT_{>MIC}$
PSA 2136	isogenic, PAO1 + GES-1	>64; 0%	8; 88%	1; 100%
PSA 2137	isogenic, PAO1 + GES-5	8; 87%	1; 100%	4; 94%
PSA 2138	isogeneic, PAO1 + GES-15	>64; 0%	4; 99%	1; 100%
PSA 2139	clinical, ST-235, GES-5, *oprD* (nt1Δ3), *mexZ* (nt292Δ11)	16; 58%	2; 100%	>64; 0%
PSA 2140	clinical, ST-235, GES-15, *oprD* (nt1Δ3), *mexZ* (nt292Δ11)	>64; 0%	16; 62%	8; 75%

GES, Guinea extended spectrum.
[a]MICs determined at a fixed avibactam concentration of 4 mg/L.
[b]$fT_{>MIC}$ expressed as the ceftazidime exposure. Avibactam $fT_{>C_T}$ of 1 mg/L = 84%.

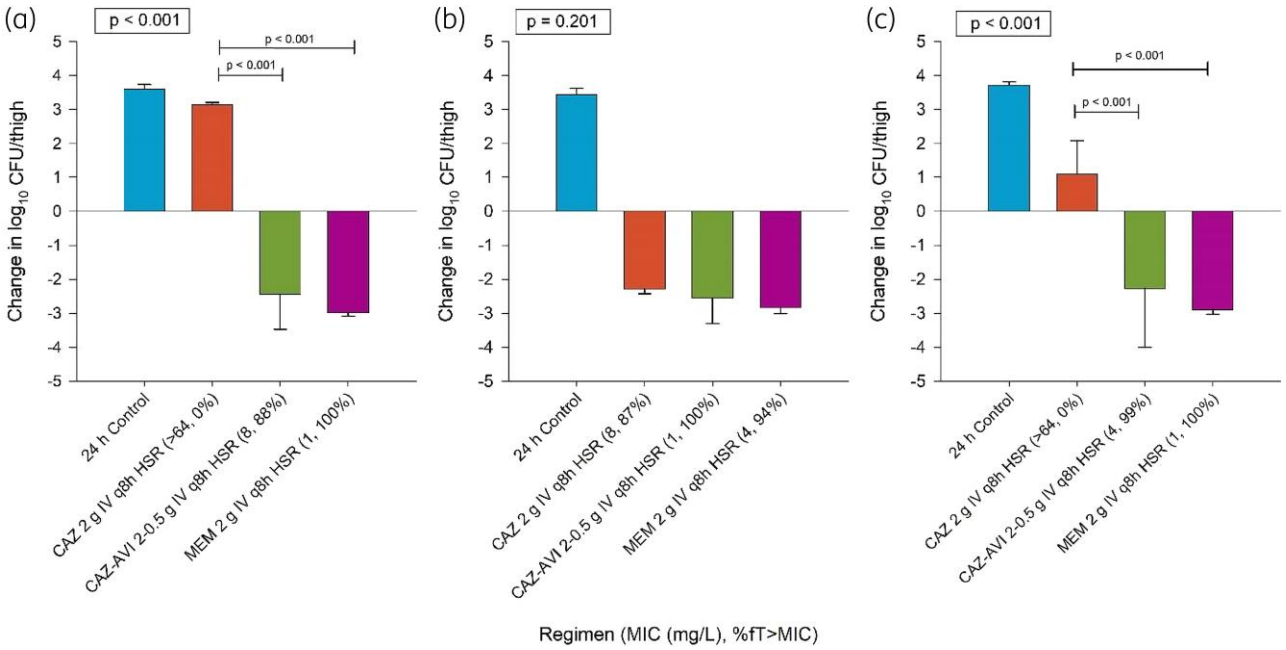

Figure 1. *In vivo* change in $\log_{10}$ cfu/thigh of each isolate treated with saline control, ceftazidime 2 g IV q8h 2 h infusion HSR, ceftazidime/avibactam 2.5 g q8h 2 h infusion HSR or meropenem 2 g q8h 3 h infusion HSR for three *P. aeruginosa* with inserted GES β-lactamases. (a) PSA 2136, with inserted GES-1 (ESBL). (b) PSA 2137, with inserted GES-5 (carbapenemase). (c) PSA 2138, with inserted GES-15. *P* values in the boxes represent the results from one-way ANOVA; *P* values are presented if between-group differences were present. The *x*-axis describes the treatment HSR [MIC (mg/L), % $fT_{>MIC}$]. CAZ, ceftazidime; CAZ-AVI, ceftazidime/avibactam; MEM, meropenem.

GES-15-harbouring *P. aeruginosa* translates to *in vivo* bacterial kill when using the high-dose, extended-infusion regimen, an observation consistent with expectations based on the pharmacodynamic profile of the 2 g q8h 3 h infusion regimen.

Indeed, the combination of ceftazidime/avibactam represents a promising agent against GES-harbouring *P. aeruginosa*, due to relatively low MICs exhibited in genotypically positive isolates. A recent multinational surveillance programme of carbapenem-resistant *P. aeruginosa* found GES as the second most common β-lactamase assessed (behind only VIM) using phenotypic (mCIM) and genotypic (CarbaR and research-use-only CarbaR NxG) methods.[3] In the 59 GES-positive isolates, 90% tested susceptible to ceftazidime/avibactam (MIC ≤8 mg/L).[3] In the present study, the ceftazidime/avibactam HSR produced bactericidal activity against all three isogenic isolates, which would be expected as the MIC of 1–8 mg/L would correspond to an $fT_{>MIC}$ of 88%–100% for the murine HSR, which would predict success for this combination, as ceftazidime reaches the 50%–70% $fT_{>MIC}$

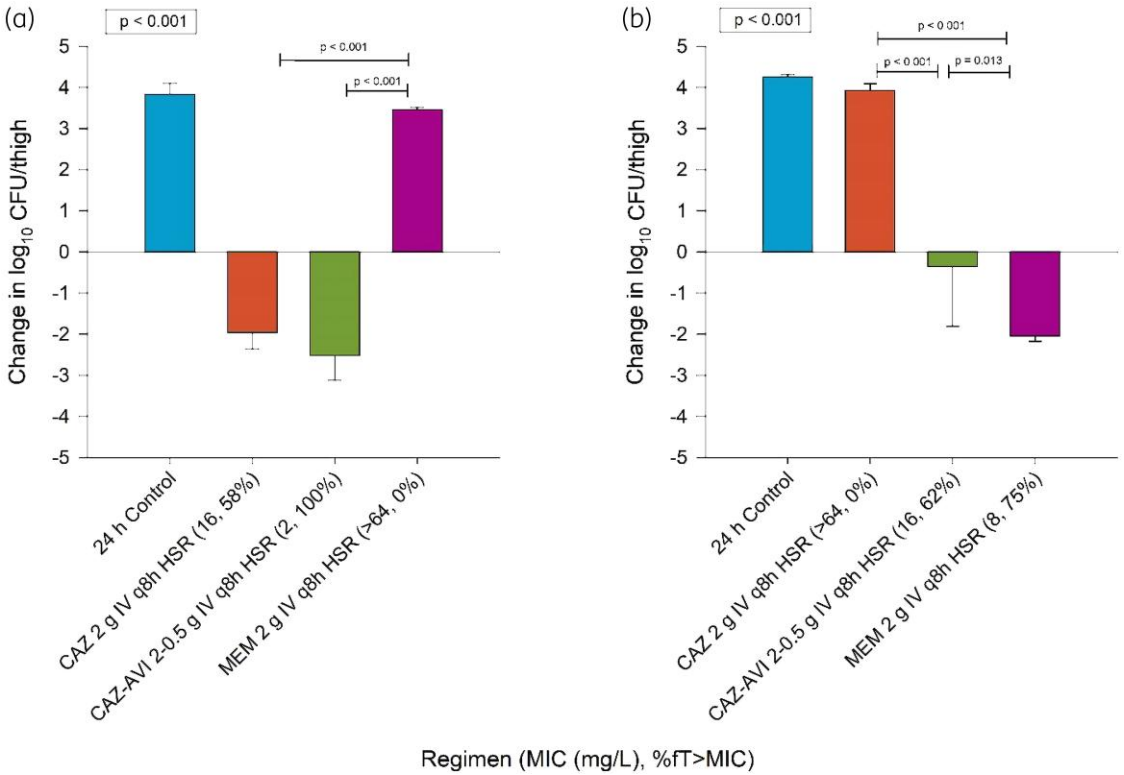

Figure 2. *In vivo* change in $\log_{10}$ cfu/thigh of each isolate treated with saline control, ceftazidime 2 g IV q8h 2 h infusion HSR, ceftazidime/avibactam 2.5 g q8h 2 h infusion HSR or meropenem 2 g q8h 3 h infusion HSR for two clinical *P. aeruginosa* with GES β-lactamases. (a) PSA 2139, clinical isolate harbouring GES-5 (carbapenemase). (b) PSA 2140, clinical isolate harbouring GES-15. *P* values in the boxes represent the results from one-way ANOVA; *P* values are presented if between-group differences were present. The *x*-axis describes the treatment HSR [MIC (mg/L), % $fT_{>MIC}$]. CAZ, ceftazidime; CAZ-AVI, ceftazidime/avibactam; MEM, meropenem.

target.[20] Interestingly, the mutational changes of GES-15 resulted in an elevated ceftazidime/avibactam MIC (16 mg/L), which resulted in bacteriostasis, despite reaching 62% $fT_{>MIC}$. Failure of ceftazidime/avibactam to reach 1 $\log_{10}$ kill against the GES-15-harbouring clinical isolate may suggest a higher pharmacodynamic target of avibactam [i.e. higher threshold concentration (C_T)] is needed in the presence of GES and/or other mechanisms (i.e. porin/efflux), although, notably, these isolates lacked AmpC derepression.[10,20,21] Studies including more isolates with various GES subtypes are needed to further assess the efficacy of the ceftazidime/avibactam HSR against different variants.

The development of resistant mutants was not assessed in the present study and future studies should assess the development of resistant GES-15-harbouring isolates upon treatment with ceftazidime/avibactam for GES-5-positive *P. aeruginosa* as seen in the clinical case (or vice versa with meropenem and GES-15). While the findings of our present study are notable, limited isolates were available for evaluation. As such, an assessment of ceftazidime/avibactam using clinically achievable exposures against a larger and more diverse population of GES-harbouring *P. aeruginosa* will better elucidate the *in vivo* activity of this novel β-lactam/β-lactamase combination. Evaluation of alternative agents (i.e. imipenem/relebactam, cefiderocol, cefepime/taniborbactam and cefepime/zidebactam)[22–25] that have been found to have *in vitro* activity against such isolates or combinations of agents is also warranted against these challenging pathogens.

In conclusion, our evaluation using the translational murine model with the incorporation of humanized exposures found differences between GES-15 and GES-5 had *in vivo* consequences consistent with the MICs. Despite increases in the meropenem MIC, an inserted GES-5 into a wild-type *P. aeruginosa* failed to cause *in vivo* microbiological failure when treated with meropenem exposures mimicking high-dose, extended-infusion therapy. Against the clinical GES-5-harbouring isolate, ceftazidime with and without avibactam produced significant bacterial kill compared with growth for meropenem, consistent with the *in vitro* profiles of each agent. Interestingly, the mutational changes of GES-15, which resulted in ceftazidime/avibactam resistance and bacteriostasis *in vivo*, restored meropenem *in vitro* activity and *in vivo* efficacy. Future studies including more isolates, other agents and combination therapy are needed to enhance our current knowledge regarding the optimal treatment for these challenging GES-producing pathogens.

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