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Efficacy of Humanized Cefiderocol Exposure Is Unaltered by Host Iron Overload in the Thigh Infection Model

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ABSTRACT Cefiderocol is a siderophore-cephalosporin conjugate with greater *in vitro* potency under iron-depleted conditions. During infection, iron is scarce in host tissue; however, it is not known whether iron overload in the host, such as in cases of hereditary hemochromatosis, alters the efficacy of cefiderocol. We compared cefiderocol efficacy between iron-overloaded and standard murine thigh infection models. Female CD-1 mice rendered neutropenic received 2 weeks of iron dextran at 100 mg/kg of body weight/day intraperitoneally (iron-overloaded model) or no injections (standard model). Mice were inoculated (10^7 CFU/ml) with *Enterobacterales*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* with previously determined cefiderocol MICs from 0.25 to 64 mg/liter. Human-simulated regimens of cefiderocol or meropenem (2 g every 8 h [q8h], 3-h infusion) were administered for 24 h (31 strains) or 72 h (15 strains; cefiderocol only). Procedures were simultaneously performed in standard and iron-overloaded models. Mean bacterial burdens ($\log_{10}$ CFU/thigh) at baseline were 5.75 ± 0.47 versus 5.81 ± 0.51 in standard versus iron-overloaded models, respectively. At 24 h, mean burdens in standard versus iron-overloaded models decreased by -0.8 ± 1.9 versus -1.2 ± 2.0 ($P = 0.25$) in meropenem-treated mice and by -1.5 ± 1.4 versus -1.6 ± 1.5 ($P = 0.54$) in cefiderocol-treated mice. At 72 h, mean burdens in cefiderocol-treated mice decreased by -2.5 ± 1.5 versus -2.5 ± 1.4. No overall differences in efficacy between the models were observed for meropenem or cefiderocol. Human-simulated exposure of cefiderocol is equally efficacious in iron-overloaded and normal hosts. The potential clinical use of cefiderocol to treat Gram-negative infections in patients with iron overload is supported.

KEYWORDS Gram-negative bacteria, animal models, cephalosporin, iron regulation, siderophores, soft-tissue infection

Cefiderocol is a siderophore-conjugated cephalosporin that is recognized by outer membrane transporters of iron in Gram-negative bacteria, facilitating uptake into the periplasmic space, its site of activity. The expression of these transporters, along with native bacterial siderophores, is upregulated when environmental iron is low (1). Evidence supporting this Trojan horse mechanism is found in measurements of the *in vitro* MIC of cefiderocol for Gram-negative bacteria in broth with various iron concentrations (2). When excess iron is added to the broth, cefiderocol MIC increases up to that of ceftazidime, which contains the same cephalosporin core structure and side chain but lacks a siderophore moiety. When iron is removed, the *in vitro* MIC of cefiderocol decreases below that observed in standard cation-adjusted Mueller-Hinton Broth, indicating increased potency of the drug when iron is not available.

Free (non-protein-bound) iron is sparse in living tissue because of its well-known propensity to catalyze the production of damaging oxygen free radicals. It is further restricted via the hypoferremic response to infection in mammalian hosts, where iron is sequestered intracellularly to deprive infectious organisms of this essential nutrient

(3). Therefore, the low availability of iron *in vivo* during infection is similar to that of iron-depleted broth *in vitro* and may contribute to the efficacy of cefiderocol. However, the hypoferremic response is mediated through hepcidin, a protein with expression under the control of the inflammatory cascade as well as overall iron status of the host (4); therefore, patients with iron overload may have a diminished hypoferremic response. Furthermore, while iron sequestration is accomplished by multiple mechanisms (5), iron overload may exceed the capacity of these pathways to restrict iron. This potential for increased bacterial iron availability, coupled with *in vitro* observations of increased MIC with excess iron, raises a concern that the efficacy of siderophore-antibiotic conjugates like cefiderocol could be impacted in the setting of host iron overload.

Several diseases can lead to chronic iron overload, including hereditary hemochromatosis, a family of disorders involving mutations to the hepcidin gene, as well as thalassemia, which necessitates chronic blood transfusions (4). Iron overload can also be caused acutely in the case of massive transfusion or pharmacological iron overdose. Recently, we developed a murine model of thigh infection with comorbid iron overload that replicates chronic deposition of iron in the liver and spleen, important organs of iron storage, as well as elevated plasma iron from acute iron overload (6). We compared the efficacy of 24 h of human-simulated plasma exposure of cefiderocol 2 g q8h (3-h infusion) in this translational iron-overloaded thigh infection model to the standard murine thigh infection model against a collection of Gram-negative bacteria, including *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, which have been studied previously in the standard model. For a subset of bacterial isolates, we extended the treatment duration in separate experimental runs up to 72 h to determine if adaptive resistance developed in the presence of iron overload.

RESULTS

Bacterial density studies. The efficacy of human-simulated exposure of cefiderocol was compared between the standard neutropenic murine thigh infection model and the iron-overloaded murine thigh infection model. To control for any effect of the iron-overloading treatment on pathogen fitness, the efficacy of a non-siderophore-conjugated antibiotic, meropenem, was also compared between models for a subset of bacterial strains. Bacterial densities (means $\pm$ standard deviations [SD]) averaged across all isolates are presented in Table 1. In the absence of antimicrobial treatment, all isolates grew adequately in both standard and iron-overloaded models.

Human-simulated meropenem exposure produced no significant difference in overall efficacy between the standard and iron-overloaded murine thigh infection models when averaged over 12 strains (Table 1). Efficacy by strain is presented in Fig. 1. Seven strains treated with the meropenem human-simulated regimen had significant differences in efficacy between the models, yet only one of these differences resulted in a categorical difference: ACB 127, which had growth in the standard model and stasis in the iron-overloaded model. No trends were observed favoring the efficacy of meropenem in either model.

In the 31 strains studied with 24 h of the cefiderocol human-simulated regimen, no significant difference in overall efficacy was observed between the models (Table 1). While placebo-dosed control mice had significantly greater overall bacterial growth at 24 h in the iron-overloaded model, both models exceeded 3 $\log_{10}$ CFU/thigh overall growth, supporting the fitness of these strains in both models. For individual isolates (Fig. 2), significant differences in efficacy between models were observed in 5/16 *Enterobacterales*, 1/8 *A. baumannii*, and 1/7 *P. aeruginosa* strains. Three of these isolates had categorical differences in efficacy between models: EC 458, with stasis in the standard model and killing in the iron-overloaded model; ACB 86, with stasis in the standard model and growth in the iron-overloaded model; and PSA 1588, with growth in the standard model and killing in the iron-overloaded model. No trends favoring cefiderocol efficacy in either model were observed.

TABLE 1 Bacterial burdens (means ± SD) in standard and iron-overloaded murine thigh infection models averaged across all strains studied

| Treatment and parameter[a] | Values (log$_{10}$ CFU/thigh) for each model | | P value |
	Standard model	Iron overloaded	
Meropenem 24-h efficacy (12 strains)			
Baseline	5.77 ± 0.45	5.92 ± 0.47	0.064
Saline (Δ)	+2.94 ± 0.99	+2.90 ± 0.77	0.833
Meropenem HSR (Δ)	−0.81 ± 1.94	−1.19 ± 2.04	0.247
Cefiderocol 24-h efficacy (31 strains)			
Baseline	5.75 ± 0.47	5.81 ± 0.51	0.227
Saline (Δ)	+3.17 ± 1.07	+3.43 ± 0.77	0.008
Cefiderocol HSR (Δ)	−1.46 ± 1.40	−1.55 ± 1.48	0.536
Cefiderocol 72-h efficacy (15 strains)			
Baseline	5.52 ± 0.44	5.54 ± 0.38	0.697
Saline 24 h (Δ)	+3.54 ± 0.77	+3.53 ± 0.71	0.905
Cefiderocol HSR 24h (Δ)	−1.77 ± 1.12	−1.95 ± 0.77	0.187
Saline 48 h (Δ)	+3.13 ± 1.15	+3.88 ± 0.47	<0.001
Cefiderocol HSR 48 h (Δ)	−2.05 ± 1.82	−2.35 ± 1.12	0.179
Saline 72 h (Δ)	+2.02 ± 1.83	+3.73 ± 0.16	0.037
Cefiderocol HSR 72 h (Δ)	−2.48 ± 1.48	−2.53 ± 1.42	0.807

[a]Burdens in saline-dosed controls and treatment groups are presented as changes from baseline. Δ, change in bacterial density relative to baseline. HSR, human-simulated regimen.

For 15 bacterial strains, additional groups of mice were treated for up to 72 h (with matching controls) to assess the development of adaptive resistance, which has been observed with other siderophore-antibiotic conjugates. Overall efficacy of the cefiderocol regimen was not significantly different among models at 24, 48, and 72 h despite higher growth of the strains in the iron overload model at 48 and 72 h (Table 1). When analyzed individually (Fig. 3–5), 4 isolates had statistically significant differences in
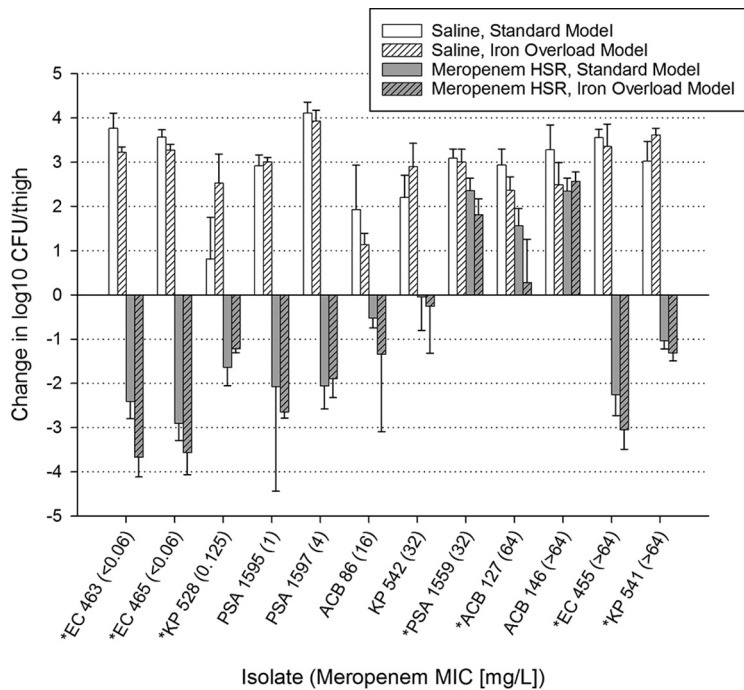
FIG 1 Efficacy of 24 h of a meropenem human-simulated regimen (HSR) (2 g q8h, 3-h infusion) against selected Gram-negative strains in the standard and iron-overloaded murine thigh infection models. Isolates for which meropenem had a significantly different change in log$_{10}$ CFU/thigh between standard and iron-overloaded models are denoted with an asterisk.
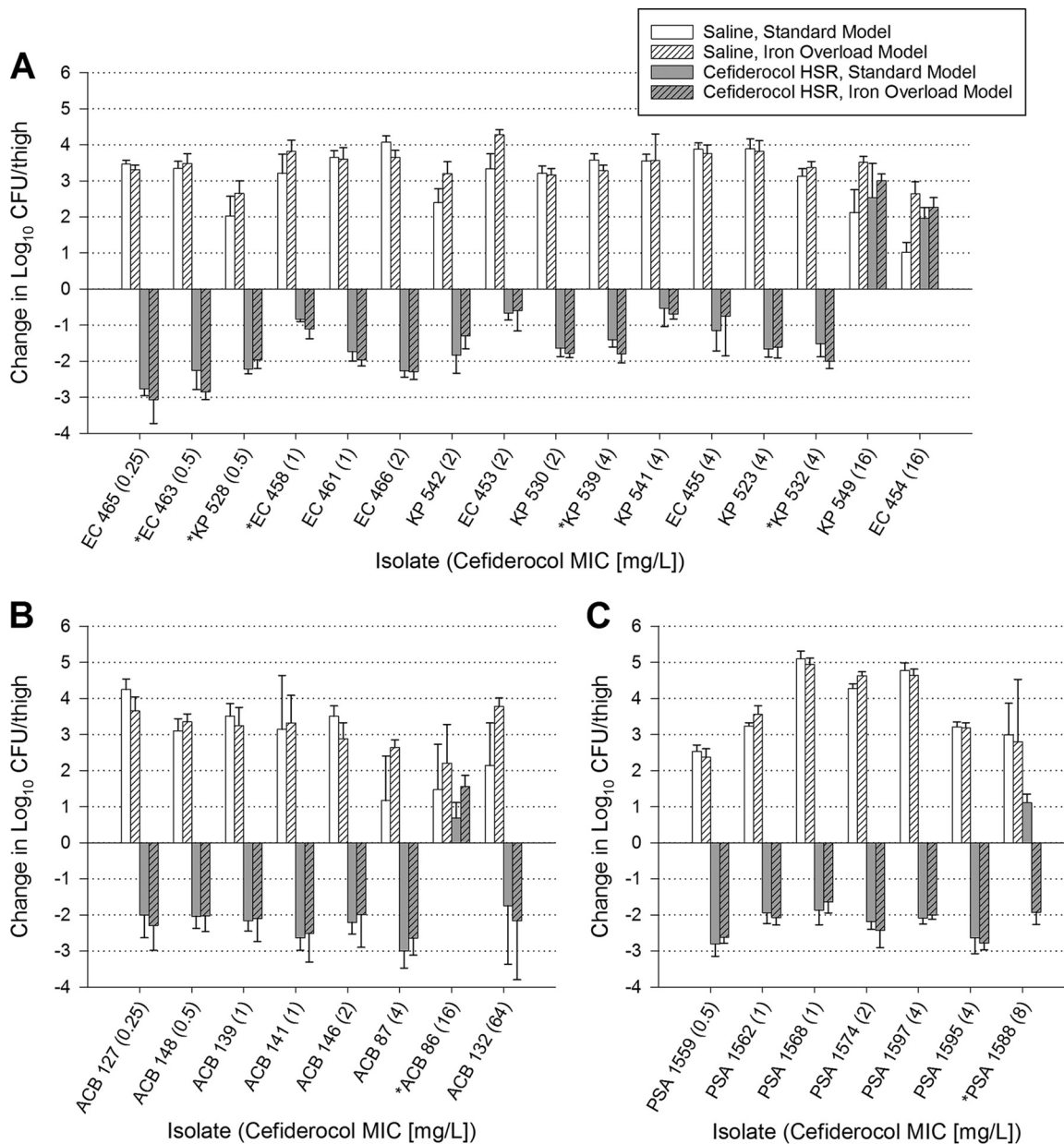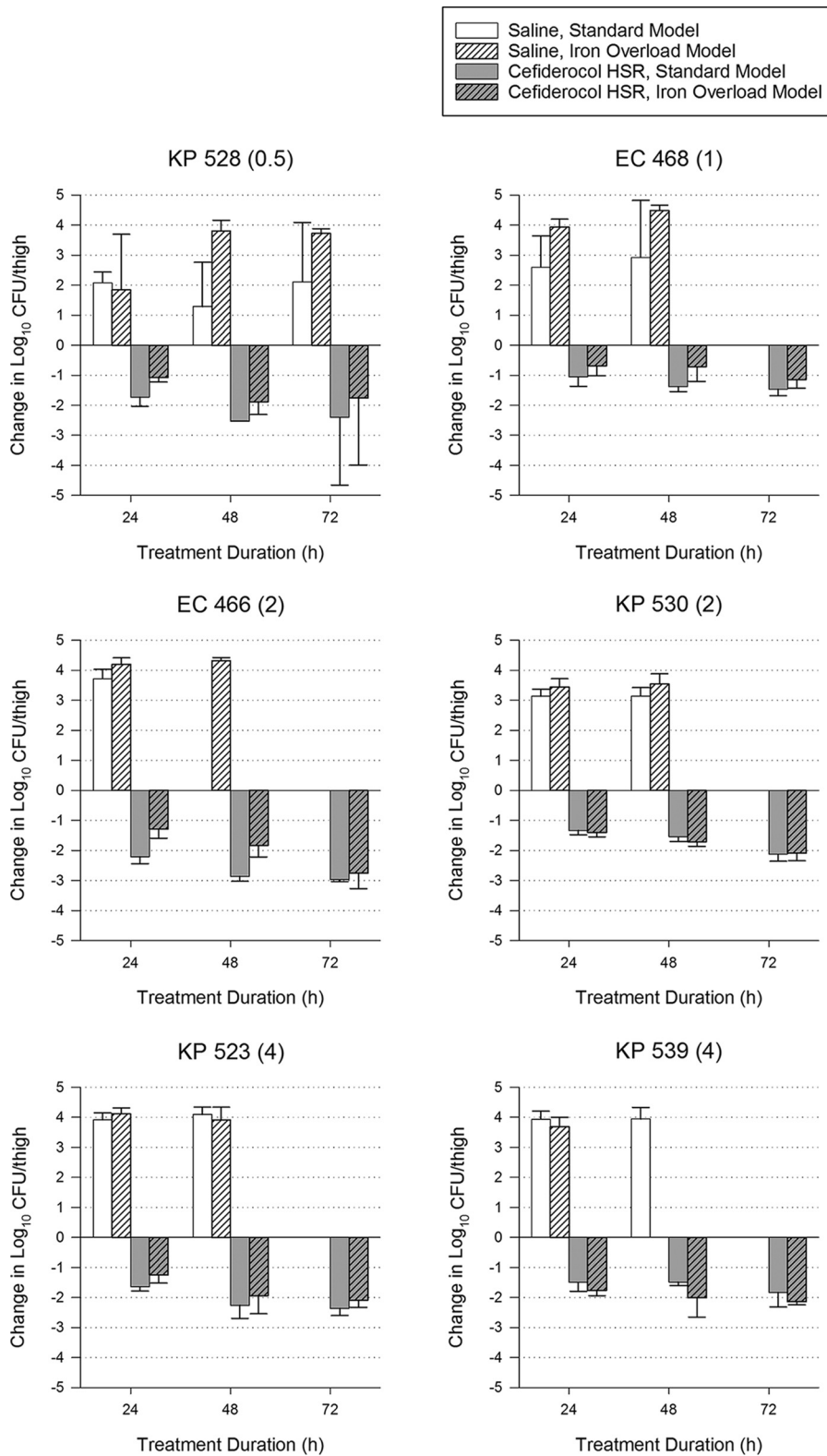
FIG 2 Efficacy of 24 h of a cefiderocol human-simulated regimen (HSR) (2 g q8h, 3-h infusion) against *Enterobacterales* (A), *A. baumannii* (B), and *P. aeruginosa* (C) in standard and iron-overloaded murine thigh infection models. Isolates for which cefiderocol had a significantly different change in $\log_{10}$ CFU/thigh between standard and iron-overloaded models are denoted with an asterisk.

efficacy at 72 h. For 2 of these isolates (ACB 139 and ACB 87), most thigh samples in both models were sterilized (<100 CFU/thigh, the lower limit of detection), indicating that maximum efficacy was achieved in both models and the differences are reflective of different baseline bacterial burdens. The only isolate with a categorical difference in efficacy between models was PSA 1588, which had growth in the standard model and killing in the iron-overloaded model, similar to observations made in the 24-h studies. No isolates in either model showed evidence of adaptive resistance, since efficacy at 72 h did not decline relative to 24-h efficacy.

DISCUSSION

This is, to our knowledge, the first study comparing the *in vivo* efficacy of a siderophore-antibiotic conjugate in a host with iron overload to a host with normal iron

FIG 3 Efficacy of 72 h of a cefiderocol human-simulated regimen (HSR) (2 g q8h, 3-h infusion) against 6 *Enterobacterales* isolates in standard and iron-overloaded murine thigh infection models. Cefiderocol MIC (mg/liter) is shown in parentheses after the isolate name. Isolates for which cefiderocol had a significantly different change in log$_{10}$ CFU/thigh at 72 h between standard and iron overload models are denoted with an asterisk.
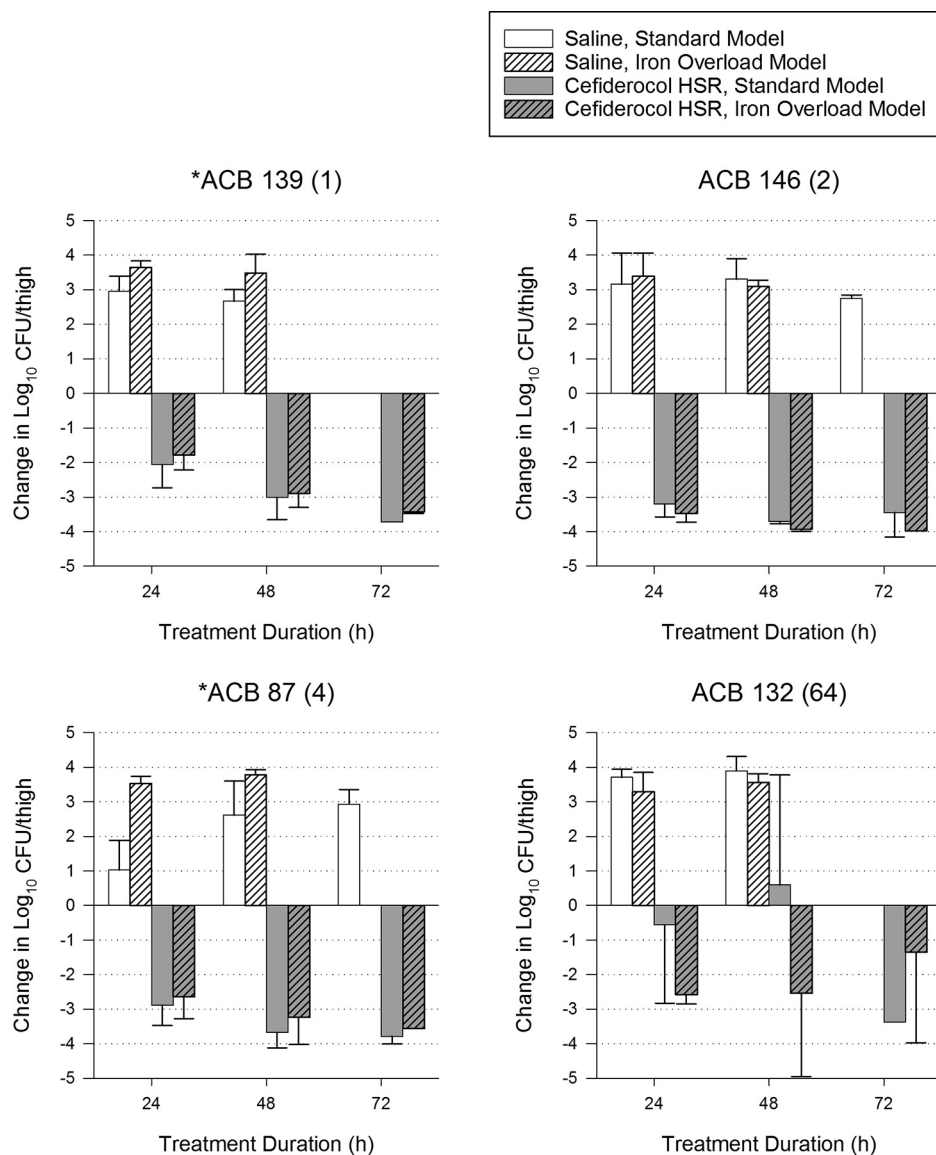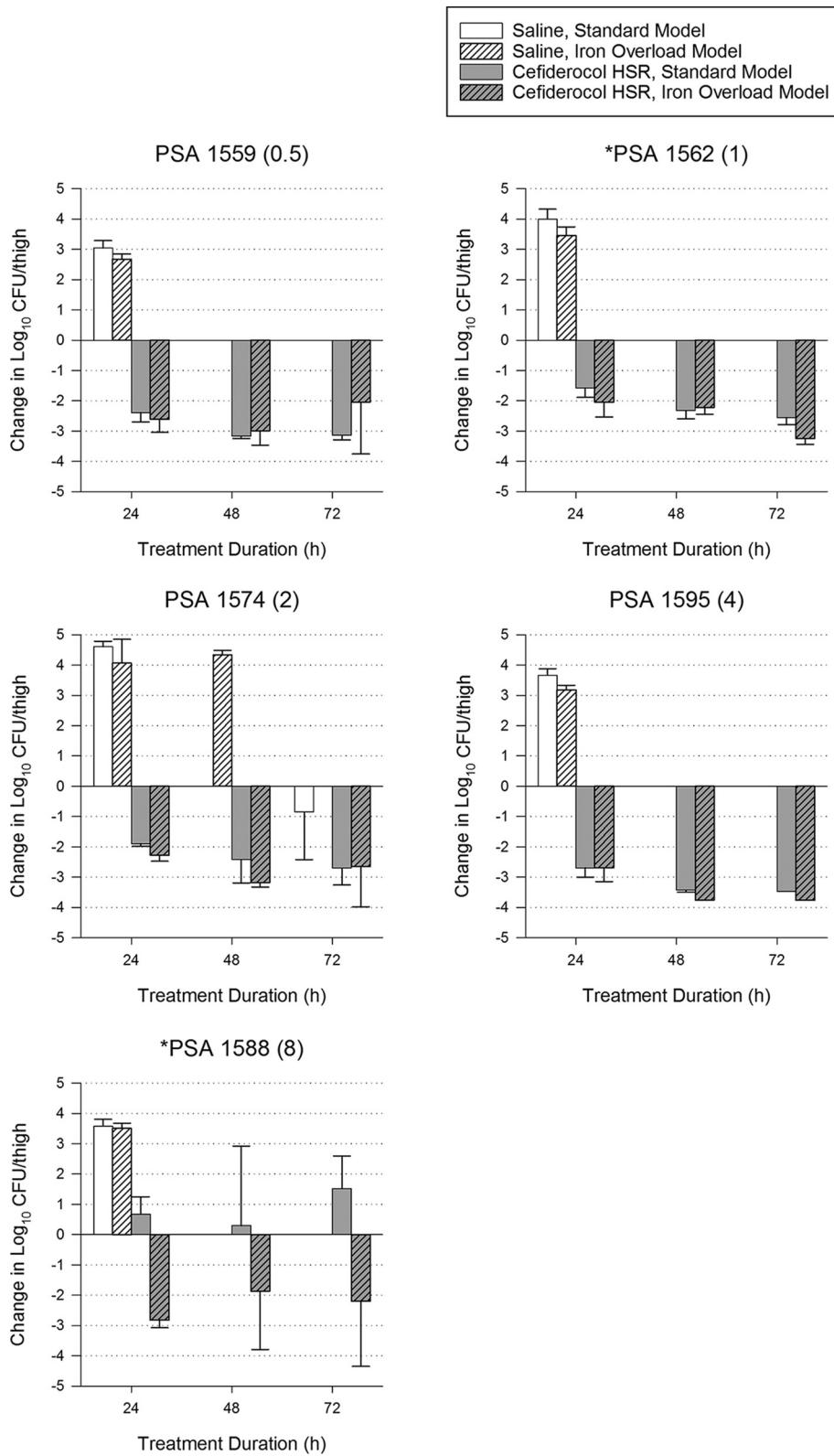
FIG 4 Efficacy of 72 h of a cefiderocol human-simulated regimen (HSR) (2 g q8h, 3-h infusion) against 4 *A. baumannii* isolates in standard and iron-overloaded murine thigh infection models. Cefiderocol MIC (mg/liter) is shown in parentheses after the isolate name. Isolates for which cefiderocol had a significantly different change in $\log_{10}$ CFU/thigh at 72 h between standard and iron overload models are denoted with an asterisk.

status. Iron overload could alter the host immune ability to restrict iron, potentially causing *in vivo* conditions similar to those of *in vitro* iron excess, which has been shown to increase the MIC of cefiderocol (2). In murine thigh and lung infection models, cefiderocol MIC as measured in iron-depleted broth was best predictive of cefiderocol efficacy, and this methodology is recommended by the Clinical and Laboratory Standards Institute (7, 8). Given the predictive value of iron-depleted MIC on *in vivo* efficacy, this preclinical investigation is necessary to determine if *in vivo* iron overload could compromise the drug's efficacy before clinical studies of cefiderocol in patients with iron overload could be performed.

In order to make a comparison between the standard and iron-overloaded models, it was important to establish that the iron overload model did not impact the fitness of the bacteria relative to the standard model and that the bactericidal activity of a non-siderophore beta-lactam was unaffected by iron overload. Iron is a regulator of

FIG 5 Efficacy of 72 h of a cefiderocol human-simulated regimen (HSR) (2 g q8h, 3-h infusion) against 5 *P. aeruginosa* isolates in standard and iron-overloaded murine thigh infection models. Cefiderocol MIC (mg/liter) is shown in parentheses after the isolate name. Isolates for which cefiderocol had a significantly different change in log_{10} CFU/thigh at 72 h between standard and iron overload models are denoted with an asterisk.

several virulence factors in *Escherichia coli* (9), *Klebsiella pneumoniae* (10), *Acinetobacter baumannii* (11), and *Pseudomonas aeruginosa* (12); therefore, the iron-overloading treatments could have altered the fitness of these organisms *in vivo* and produced variation in response to antibiotics. Bacterial fitness, as measured by growth in the absence of treatment, was equivalent in both models for all species studied. In addition, meropenem was equally efficacious in both models. Therefore, any differences in cefiderocol efficacy between the models could be attributed to the siderophore-conjugated antibiotic itself rather than differences between the models or general responsiveness to beta-lactams.

Cefiderocol efficacy was not diminished by clinically relevant host iron overload. Liver iron deposition in the iron overload model is within the range seen in patients with hereditary hemochromatosis, while the plasma iron concentrations exceed thresholds of acute iron toxicity (13, 14). Furthermore, the plasma iron content in this model exceeds the total iron binding capacity in mice (15), suggesting that bacteria could have been exposed to excess free iron, similar to conditions which increased cefiderocol MIC *in vitro*. Under these conditions representing extremes of iron overload, most strains had no significant difference in response to cefiderocol, and among those that did, only one exceeded 0.5 $\log_{10}$ CFU/thigh, which is generally regarded as the magnitude of difference considered clinically meaningful in the murine thigh infection model. In a further assessment of possible clinical significance, only 3 strains had a categorical difference in efficacy between models at 24 h, of which 2 had greater efficacy in the setting of iron overload. The duration of treatment did not impact these conclusions: with 72 h of human-simulated cefiderocol exposure, there was no evidence of diverging efficacy between the models.

Our work builds upon prior studies in the standard neutropenic murine thigh infection model using the same isolates. Monogue and colleagues studied the same human-simulated regimen of cefiderocol over 24 h in a larger set of *Enterobacterales*, *P. aeruginosa*, and *A. baumannii* isolates, finding consistent activity of approximately 1 to 2 $\log_{10}$ killing against isolates with cefiderocol MIC of ≤4 mg/liter (16). The present results from the 24-h standard thigh infection model reproduce these observations with remarkable agreement on the magnitude of killing for individual isolates between both studies. Additional investigation on a subset of these isolates using 72 h of human-simulated cefiderocol exposure was performed by Stainton and colleagues, finding that during an extended treatment period, efficacy was sustained, and only one isolate had a significant (two 2-fold dilutions) increase in postexposure cefiderocol MIC, which the authors interpreted as evidence against adaptive resistance (17). Three isolates (KP 539, ACB 87, and PSA 1595) from this study were included in the present experiments, where similar magnitudes of killing at 72 h were seen in both standard and iron-overloaded models.

Only one isolate studied showed a major difference in response to cefiderocol exposure between the models: PSA 1588 was killed by 2 $\log_{10}$ CFU/thigh in the iron-overloaded model but grew approximately 1 $\log_{10}$ CFU/thigh in the standard model. After observing this unexpected response in the 24-h experiments, the isolate was included in the 72-h experiments, where the findings were replicated and sustained over the 72-h treatment period. When previously studied by Monogue and colleagues, this isolate grew in the standard murine thigh infection model as observed presently. The reason for the enhanced efficacy of cefiderocol against this isolate in the iron-overloaded model is unknown and warrants further investigation, given the complex role of iron in the regulation of *P. aeruginosa* virulence (9, 18).

In summary, human-simulated cefiderocol exposure was similarly efficacious against a large collection of Gram-negative bacteria with a wide range of cefiderocol MICs regardless of the presence of host iron overload. No evidence was found to support the hypothesis that diminished *in vitro* activity of cefiderocol in the presence of excess iron could be translated to the iron-overloaded host. Cefiderocol efficacy in both models against particular isolates closely matched previous studies

at both the 24-h and 72-h endpoints. This fact, along with the similar responses in both models to a non-siderophore beta-lactam, improves the validity of these findings. Clinical study of cefiderocol in patients with disorders of iron overload is supported.

MATERIALS AND METHODS

Antimicrobial test agents. Cefiderocol (S-649266) 500-mg vials (lot 12M01) were provided by Shionogi (Osaka, Japan). Commercially available meropenem 500-mg vials (lot 4B18F05; Fresenius Kabi USA, Lake Zurich, IL) were obtained from Cardinal Health (Dublin, OH). Vials were reconstituted with 0.9% sodium chloride (normal saline; NS) per manufacturer instructions and then further diluted with NS to the final dosing concentrations.

Bacterial isolates. Thirty-one clinical Gram-negative isolates, including 16 *Enterobacterales*, 7 *P. aeruginosa*, and 8 *A. baumannii* isolates, were studied. MICs to cefiderocol in iron-depleted cation-adjusted Mueller-Hinton broth (CAMHB) and meropenem in standard CAMHB for each isolate were previously determined in triplicate (17); cefiderocol MICs ranged from 0.25 to 16 mg/liter in *Enterobacterales*, 0.5 to 8 mg/liter in *P. aeruginosa*, and 0.25 to 64 mg/liter in *A. baumannii*. All isolates were previously frozen in skim milk at −80°C. Bacterial suspensions were prepared from the second of two transfers on Trypticase soy agar plates with 5% sheep blood (TSA II; Becton, Dickinson, & Co., Sparks, MD, USA), which were incubated for 18 to 24 h at 37°C.

Animals. Animal usage was approved by the Hartford Hospital Institutional Animal Care and Use Committee (assurance number A3185-01). Specific-pathogen-free outbred female mice (CD-1; Charles River Laboratories, Wilmington, MA) were allowed to acclimate for 48 h after arrival at the facility before study procedures began. Groups of 3 mice were housed together in HEPA-filtered cages (Innovive, San Diego, CA) with paper nesting material for enrichment; food and water were available *ad libitum*. Controlled room temperature and a 12-h light/dark cycle were employed. During study procedures, mice were monitored at least 3 times daily for signs of morbidity and were euthanized via CO_2 asphyxiation followed by cervical dislocation if moribund.

Neutropenic thigh infection models. The standard murine thigh infection model was utilized as previously described (16, 17). In brief, neutropenia was produced with 150 mg/kg cyclophosphamide at day 4 and 100 mg/kg at day 1 prior to inoculation, and 5 mg/kg uranyl nitrate was used at day 3 prior to inoculation to decrease renal elimination of the study compounds. In addition, an iron-overloaded murine thigh infection model was used in which mice received the treatments described above plus 14 days of iron dextran (100 mg/kg/day intraperitoneally). This model has been shown to produce supranormal iron concentrations in plasma, liver, and spleen (6). In both models, mice were intramuscularly inoculated in both thighs with 0.05 ml of bacterial suspensions of approximately 10^7 CFU/ml.

Bacterial density studies. All experiments were performed in the standard murine thigh infection model and the iron-overloaded murine thigh infection model simultaneously. After inoculation, mice were randomized into baseline (0 h), growth control, or treatment groups with 3 mice (6 thighs) each. Two hours after inoculation (0 h), mice in baseline groups were harvested, while mice in treatment or growth control groups began receiving subcutaneous doses of antimicrobial test agents or NS placebo injections, respectively.

A cefiderocol dosing regimen in mice that simulates the human free cefiderocol plasma profile after clinical doses of 2 g q8h (3-h infusion) was studied (19). The human-simulated regimen has been shown to produce similar exposures in standard and iron-overloaded murine thigh infection models (6) and consisted of cefiderocol doses of 15 mg/kg, 20 mg/kg, 25 mg/kg, 10 mg/kg, and 5 mg/kg given at 0, 1, 2, 4, and 6 h, respectively. In addition, a meropenem regimen shown to simulate free plasma exposure in both standard and iron-overloaded models after clinical doses of 2 g q8h (3-h infusion) was utilized, consisting of doses of 65 mg/kg, 65 mg/kg, 45 mg/kg, and 45 mg/kg given at 0, 1.25, 3.5, and 6 h, respectively (6, 16). Both human-simulated regimens were given every 8 h over the treatment period.

At the scheduled time of harvest, mice were euthanized via CO_2 asphyxiation followed by cervical dislocation, after which both thighs were aseptically harvested, homogenized in NS, and plated to determine bacterial CFU/thigh. Mice which succumbed to infection prior to the scheduled time of harvest were included in the next group due for harvest.

Data analysis. Efficacy was defined as the change in log_{10} CFU/thigh at the end of the treatment period compared with 0-h controls in the same model and from the same experimental run. The overall efficacy of a treatment was defined as the mean efficacy for all isolates receiving that treatment and was calculated using data from individual thighs. Comparisons of overall efficacy and efficacy by isolate were made between the standard and iron-overloaded models using Student's *t* test, with two-tailed *P* values of ≤0.05 deemed significant. For individual isolates with a statistically significant difference in efficacy between the models, comparisons were also made based on categorical efficacy, with killing defined as a decrease of ≥1 log_{10} CFU/thigh, growth defined as an increase of the same magnitude, and stasis defined as a change of <1 log_{10} CFU/thigh in either direction.

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