

Efficacy of Humanized Carbapenem and Ceftazidime Regimens against *Enterobacteriaceae* Producing OXA-48 Carbapenemase in a Murine Infection Model

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Enterobacteriaceae producing the OXA-48 carbapenemase are emerging worldwide, leaving few treatment options. Efficacy has been demonstrated *in vivo* with ceftazidime against a ceftazidime-susceptible OXA-48 isolate but not with imipenem despite maintaining susceptibility. The relationship between phenotype and *in vivo* efficacy was assessed for OXA-48 producers using humanized regimens of 2 g doripenem every 8 h (q8h; 4 h infusion), 1 g ertapenem q24h, 2 g ceftazidime q8h (2 h inf), and 500 mg levofloxacin q24h. Each regimen was evaluated over 24 h against an isogenic pair (wild-type and OXA-48 *Klebsiella pneumoniae* strains) and six clinical OXA-48 isolates with and without other extended-spectrum β -lactamases in immunocompetent and neutropenic murine thigh infection models. Efficacy was determined using the change in bacterial density versus 24-h growth controls in immunocompetent studies and 0-h controls in neutropenic studies. Bacterial reductions of ≥ 1 log CFU were observed with all agents for the wild-type strain. Consistent with low MICs, ceftazidime and levofloxacin exhibited efficacy against the isogenic OXA-48 strain, whereas doripenem did not, despite having a susceptible MIC; no activity was observed with ertapenem, consistent with a resistant MIC. Similar trends were observed for the clinical isolates evaluated. Ceftazidime, levofloxacin, and ertapenem efficacy against isogenic and clinical OXA-48-producing strains correlated well with phenotypic profiles and pharmacodynamic targets, whereas efficacy with doripenem was variable over the MIC range studied. These data suggest that carbapenems may not be a reliable treatment for treating OXA-48 producers and add to previous observations with KPC and NDM-1 suggesting that genotype may better predict activity of the carbapenems than the phenotypic profile.

Carbapenem resistance among the *Enterobacteriaceae* has been on the rise worldwide over the last decade, commonly mediated by Ambler class A β -lactamases such as KPC, as well as Ambler class B metallo- β -lactamases, including VIM, IMP, and NDM-1 (1). However, carbapenem resistance secondary to the Ambler class D β -lactamase OXA-48 has also increasingly been reported since it was first isolated in Turkey in 2001 (1–3). While OXA-48 exhibits low-level hydrolysis of carbapenems, it does not significantly hydrolyze the extended-spectrum cephalosporins, ceftazidime and cefepime (3, 4). Consistent with *in vitro* hydrolysis data, an *in vivo* dose ranging study in an immunocompetent murine peritonitis model resulted in efficacy with ceftazidime against one ceftazidime-susceptible OXA-48-producing isolate and poor activity with imipenem, despite the isolate being imipenem susceptible (5).

Discordance between *in vitro* MICs and *in vivo* efficacy with humanized regimens of carbapenems has also recently been demonstrated for KPC- and NDM-1-producing *Enterobacteriaceae* (6, 7). As such, an evaluation of this relationship using human simulated regimens of carbapenems and comparator agents for OXA-48 producers *in vivo* is warranted to assist in determining the clinical utility of these agents. In order to evaluate the consistency between pharmacodynamic targets and *in vivo* efficacy of these agents when used for OXA-48-producing isolates, the *in vivo* efficacy of human simulated regimens of doripenem, ertapenem, ceftazidime, and levofloxacin was characterized against both isogenically constructed and clinical OXA-48-producing *Enterobacteriaceae* in a murine thigh infection model.

MATERIALS AND METHODS

Antimicrobial test agents. Commercially available ertapenem (Invanz, Merck & Co., Inc., Whitehouse Station, NJ), doripenem (Doribax; Janssen Pharmaceuticals, Inc., Raritan, NJ), ceftazidime (Fortaz; GlaxoSmithKline, Philadelphia, PA), and levofloxacin (Sagent Pharmaceuticals, Schaumburg, IL) were obtained from the pharmacy department at Hartford Hospital. Each antibiotic was reconstituted according to the manufacturer's prescribing information and further diluted in normal saline to the concentrations required for dosing. Prior to each experiment, dosing solutions were prepared and stored under refrigeration until the time of use and were discarded after 24 h.

Bacterial isolates and susceptibility testing. An isogenic pair consisting of a wild-type *Klebsiella pneumoniae* strain (KP 454) and an OXA-48 strain derived by conjugation using antibiotic-containing plates, as well as two clinical OXA-48-producing *K. pneumoniae* isolates (KP 452 and KP 453), were provided by P. Nordmann, Hospital Bicêtre (Bicêtre, France). Ertapenem and doripenem MICs for these isolates were determined via Etest (AB bioMérieux, Solna, Sweden) according to the manufacturer's instructions, while MICs for ceftazidime and levofloxacin were determined using broth microdilution consistent with the Clinical

and Laboratory Standards Institute (CLSI) methodology (8). *Pseudomonas aeruginosa* ATCC 27853 was used as the quality-control strain for the two agents, with MICs determined by broth microdilution. MIC determinations were conducted in triplicate and are reported as the modal MIC. Four additional clinical OXA-48-producing *Enterobacteriaceae* isolates (KP 464, KP 465, EC 418, CF 32) were kindly provided by C. Lascols, International Health Management Associates, Inc. (Schaumburg, IL) (9). MICs for these isolates to all four test agents were determined using broth microdilution as outlined above. Isolates were screened for the presence of KPC, OXA-48-like, VIM, IMP, and NDM carbapenemases, as well as for extended-spectrum β -lactamases (TEM, SHV, and CTX-M) and AmpC β -lactamases (CMY, DHA, FOX, MOX, ACC, and ACT) as previously described (9). All isolates were stored in double-strength skim milk (Remel, Lenexa, KS) at -80°C . Prior to all *in vivo* experiment, each isolate was subcultured twice onto Trypticase soy agar with 5% sheep blood (BD Biosciences, Sparks, MD) and incubated for 18 to 24 h at 35°C .

Immunocompetent thigh infection model. This study was reviewed and approved by the Institutional Animal Care and Use Committee at Hartford Hospital, Hartford, CT. Pathogen-free ICR mice weighing 20 to 22 g were acquired from Harlan Laboratories (Indianapolis, IN). All animals were maintained in accordance with the National Research Council's recommendations and were provided food and water *ad libitum*. An intraperitoneal injection of uranyl nitrate (5 mg/kg of body weight) was given 3 days prior to inoculation for each experiment to slow antibiotic clearance and produce a predictable level of renal impairment (10). Two hours prior to the initiation of antimicrobial therapy, both thighs of each animal were inoculated intramuscularly with 0.1 ml of an inoculum solution containing 10^8 CFU/ml of the test isolate in normal saline.

Neutropenic thigh infection model. In order to best characterize the achievable efficacy of each agent tested, the isogenic pair (wild-type and OXA-48-producing strains) and three clinical OXA-48-producing strains were also evaluated in a neutropenic infection model for comparison. All mice utilized in the neutropenic studies underwent the same procedures as described for the immunocompetent model but were also given intraperitoneal injections of 100 and 150 mg cyclophosphamide/kg (Baxter, Deerfield, IL) 1 and 4 days prior to inoculation, respectively, to induce neutropenia (10). In addition, the inoculum solution used to produce thigh infection in the neutropenic model was reduced to 10^7 CFU/ml.

***In vivo* efficacy.** Human-simulated regimens of 2 g doripenem intravenously (i.v.) every 8 h as a 4-h infusion, 1 g ertapenem i.v. every 24 h, 2 g ceftazidime i.v. every 8 h as a 2-h infusion, and 500 mg levofloxacin every 24 h were previously developed and validated by our group to achieve the human pharmacodynamic profile associated with each of these dosing regimens and were utilized throughout the course of the *in vivo* experiments (11–14). Beginning 2 h after inoculation, groups of three mice were administered a human simulated regimen of doripenem, ertapenem, ceftazidime, or levofloxacin for each test isolate. All doses for the humanized regimens were administered as 0.2-ml subcutaneous injections; dosing regimens were repeated as necessary to complete 24 h of therapy. For each isolate evaluated, groups of three control animals were given normal saline in the same volume, route, and frequency as the most frequently administered antibiotic regimen over 24 h. One group of untreated control mice was harvested at 0 h, while the control group receiving normal saline and all treated mice were harvested at 24 h. Mice that failed to survive to 24 h were harvested at the time of expiration and were included in the final data analysis. Harvesting consisted of euthanization by CO_2 exposure followed by cervical dislocation. Thighs were removed from sacrificed animals, homogenized individually in normal saline, serially diluted, and plated onto Trypticase soy agar with 5% sheep blood for determination of bacterial density. Efficacy was calculated as the change in bacterial density (in \log_{10} CFU) in treated mice after 24 h from the starting bacterial densities in 0-h control animals for all experiments conducted in neutropenic mice. In order to control for the effect of the host and associated

TABLE 1 Phenotypic and genotypic profiles of wild-type and OXA-48-producing *Enterobacteriaceae* isolates utilized in the *in vivo* efficacy studies

Isolate	Known β -lactamase content (9)	MIC ($\mu\text{g/ml}$) ^a			
		CAZ	LEV	DOR	ETP
<i>K. pneumoniae</i> 454	None	0.25	≤ 0.06	0.03	0.012
<i>K. pneumoniae</i> 454 with OXA-48 plasmid	OXA-48	0.25	≤ 0.125	0.38	3
<i>K. pneumoniae</i> 452	OXA-48	0.5	4	3	4
<i>Citrobacter freundii</i> 32	OXA-48, AmpC, CMY	1	≤ 0.03	1	1
<i>E. coli</i> 418	OXA-48, CTX-M-15	64	>32	2	16
<i>K. pneumoniae</i> 465	OXA-48, CTX-M-15	64	16	2	4
<i>K. pneumoniae</i> 453	OXA-48, CTX-M-15, TEM-1, OXA-1	>128	>32	6	2
<i>K. pneumoniae</i> 464	OXA-48, TEM, SHV	>128	>32	8	64

^a DOR, doripenem; ETP, ertapenem; CAZ, ceftazidime; LEV, levofloxacin.

variability in bacterial density of growth controls between isolates in the immunocompetent model, efficacy was calculated as the change in bacterial density in treated mice after 24 h compared to that in the 24-h immunocompetent control mice (6, 15).

RESULTS

Bacterial isolates. A wild-type *K. pneumoniae* strain (KP 454) and its derived isogenic OXA-48 strain, in addition to six clinical OXA-48-producing *Enterobacteriaceae* isolates, were utilized in the *in vivo* experiments. Phenotypic profiles for the antimicrobials evaluated and the known genotypic profiles for these isolates are described in Table 1 (9).

***In vivo* efficacy.** The isogenic wild-type and OXA-48-producing strains as well as all six clinical OXA-48-producing isolates were evaluated in the immunocompetent model. The mean (\pm standard deviation) bacterial density for control mice at 0 h was $7.05 \pm 0.30 \log_{10}$ CFU/ml in immunocompetent mice and was maintained at a mean of $6.41 \pm 0.62 \log_{10}$ CFU/ml after 24 h. The efficacy results of the immunocompetent studies are shown in Fig. 1. Bacterial reductions $\geq 1 \log$ CFU were observed with all agents tested against the wild-type strain. Consistent with phenotypic profile, ceftazidime and levofloxacin also exhibited efficacy against the isogenic OXA-48 strain, whereas doripenem did not, despite having an MIC of 0.38 $\mu\text{g/ml}$; no activity was observed with ertapenem as anticipated given an MIC of 3 $\mu\text{g/ml}$. Furthermore, $\geq 1 \log$ CFU bacterial reductions were also achieved with the humanized ceftazidime regimen for the two clinical isolates with MICs of $\leq 1 \mu\text{g/ml}$ (100% *fT*>MIC [time that the free drug concentration remains above the MIC during the dosing interval]) and one isolate with an MIC of 64 $\mu\text{g/ml}$ (0% *fT*>MIC); observed reductions were less for the remaining three clinical strains, all with MICs of $\geq 64 \mu\text{g/ml}$. The simulated levofloxacin regimen produced a greater-than-2-log CFU reduction for the clinical isolate with an MIC of $\leq 0.03 \mu\text{g/ml}$ (area under the curve [AUC]/MIC $\geq 1,467$), while little to no efficacy was demonstrated for the five isolates with MICs of $\geq 4 \mu\text{g/ml}$ (AUC/MIC ≤ 11). Consistent with an MIC of $\geq 1 \mu\text{g/ml}$ ($\leq 30\%$ *fT*>MIC), the simulated dose of ertapenem did not result in substantial bacterial reductions for any of the clinical strains. While the observed efficacy for ceftazidime, levofloxacin, and ertapenem against the six clinical isolates correlated well with phenotypic profiles and pharmacodynamic targets, variable levels of bacterial reductions (range, 0.4 to

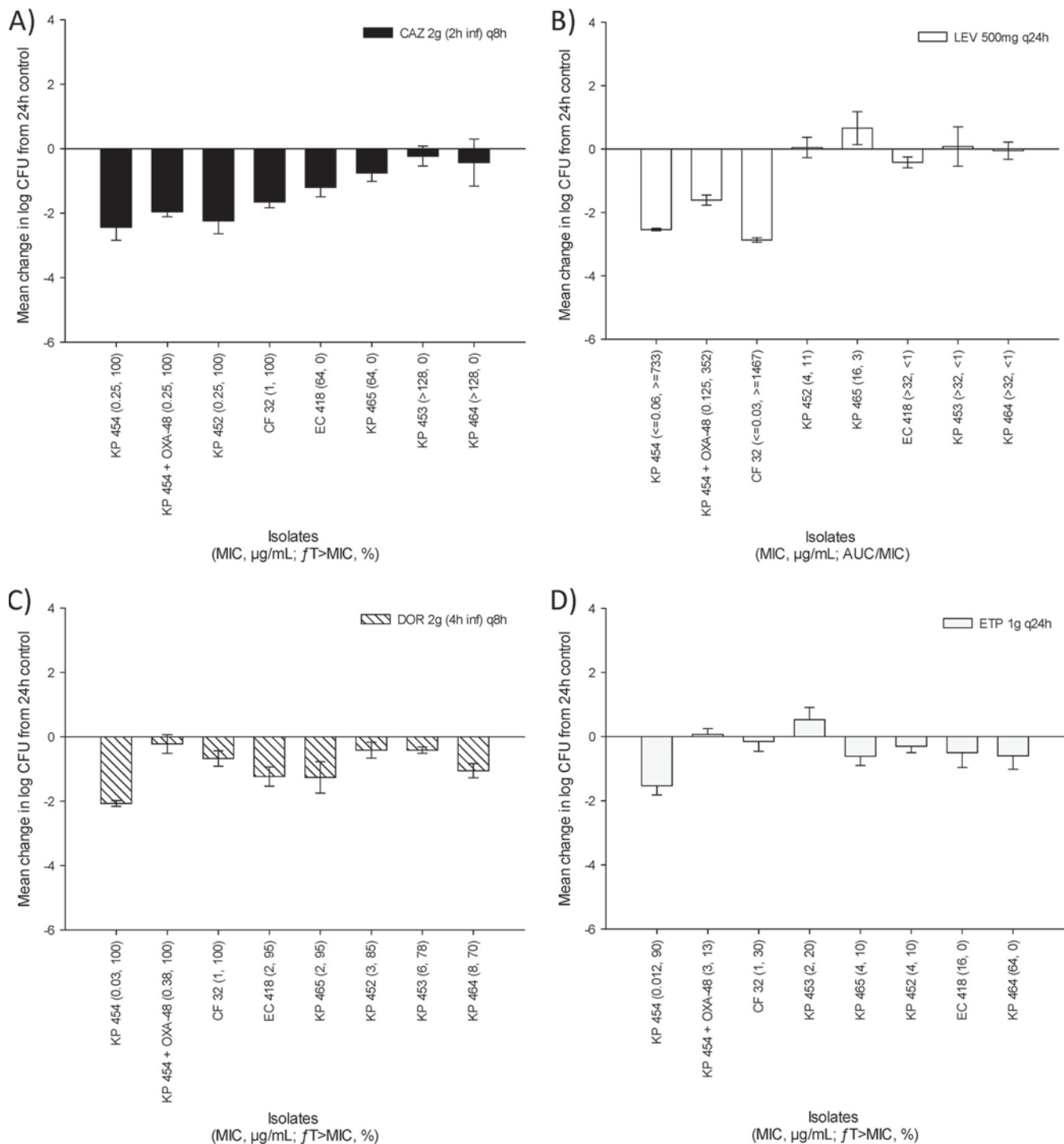


FIG 1 Efficacy of human simulated regimens of 2 g ceftazidime i.v. every 8 h as a 2-h infusion (A), 500 mg levofloxacin i.v. every 24 h (B), 2 g doripenem every 8 h as a 4-h infusion (C), and 1 g ertapenem every 24 h (D) against a distribution of OXA-48-producing *Enterobacteriaceae* isolates in an immunocompetent murine thigh infection model. Error bars represent standard deviations. Clinical and Laboratory Standards Institute susceptibility breakpoints: ceftazidime, ≤4 μg/ml; levofloxacin, ≤2 μg/ml; doripenem, ≤1 μg/ml; and ertapenem, ≤0.5 μg/ml (8).

1.3 log CFU) were noted with the humanized high-dose, prolonged-infusion doripenem regimen for the six clinical strains with MICs of ≤8 μg/ml ($fT>MIC \geq 70\%$).

In the neutropenic model, the mean bacterial density for control mice at 0 h was $5.85 \pm 0.46 \log_{10}$ CFU/ml and increased to a

mean of $8.23 \pm 0.53 \log_{10}$ CFU/ml after 24 h. The efficacy results of the neutropenic studies are shown in Fig. 2. Similar trends in efficacy with respect to phenotypic profiles and pharmacodynamic targets were noted for ceftazidime, levofloxacin, and ertapenem between immunocompetent and neutropenic studies. Efficacy

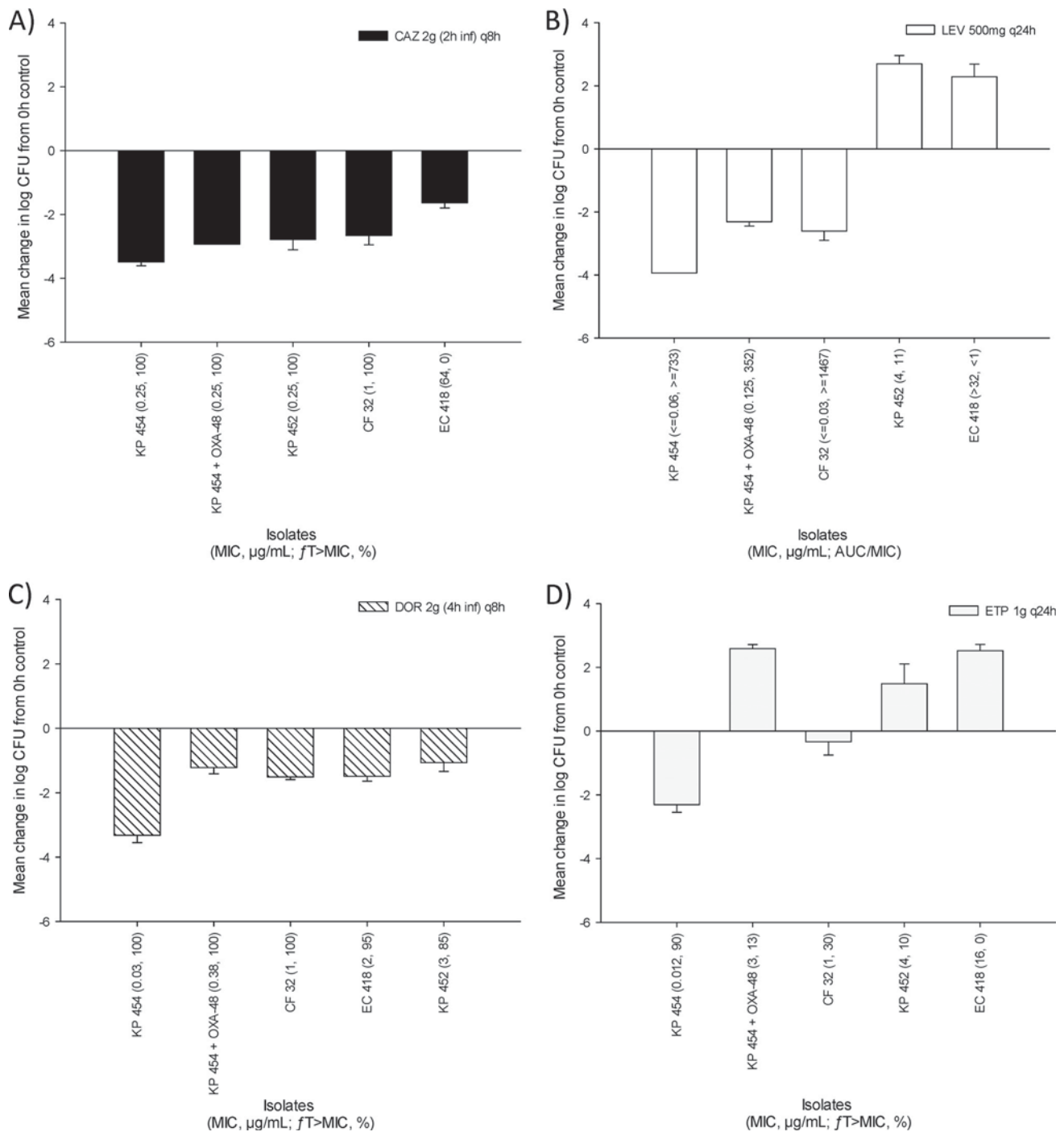


FIG 2 Efficacy of human simulated regimens of 2 g ceftazidime i.v. every 8 h as a 2-h infusion (A), 500 mg levofloxacin i.v. every 24 h (B), 2 g doripenem every 8 h as a 4-h infusion (C), and 1 g ertapenem every 24 h (D) against a distribution of OXA-48-producing *Enterobacteriaceae* isolates in a neutropenic murine thigh infection model. Error bars represent standard deviations. Clinical and Laboratory Standards Institute susceptibility breakpoints: ceftazidime, ≤ 4 $\mu\text{g/ml}$; levofloxacin, ≤ 2 $\mu\text{g/ml}$; doripenem, ≤ 1 $\mu\text{g/ml}$; and ertapenem, ≤ 0.5 $\mu\text{g/ml}$ (8).

cacy was observed with doripenem against the isogenic and three clinical OXA-48-producing strains evaluated in the neutropenic model, all with MICs of ≤ 3 $\mu\text{g/ml}$ ($fT > MIC$ of $\geq 85\%$ for all isolates). However, the magnitude of bacterial reduction was much less than what was observed with doripenem against the wild-type strain.

DISCUSSION

Carbapenem resistance due to the Ambler class D β -lactamase OXA-48 is increasing among *Enterobacteriaceae*. Previous work by our group has demonstrated widely different levels of efficacy with carbapenem monotherapy against isolates producing class A carbapenemases (KPC) versus class B metallo- β -lactama-

ses (NDM-1) and often inconsistent with previously defined pharmacodynamic targets (6, 7). Therefore, it is essential to also characterize the *in vivo* efficacy of human simulated regimens of carbapenems for *Enterobacteriaceae* producing the OXA-48 carbapenemase, as each of these enzymes appear to possess various levels of carbapenem hydrolysis (1, 16). A humanized ceftazidime regimen was also evaluated in this study, as it has previously demonstrated *in vivo* efficacy against one OXA-48-producing *K. pneumoniae* isolate in a murine peritonitis model (5). Levofloxacin was included as an additional comparator to provide further validation of these data, as its pharmacodynamic profile has previously been well characterized in the murine thigh infection model utilized (4, 17).

The observed efficacy for ceftazidime, levofloxacin, and ertapenem against the isogenic and six clinical OXA-48-producing isolates evaluated in both immunocompetent and neutropenic models correlated well with pharmacodynamic targets and *in vitro* MIC. Namely, levofloxacin and ertapenem resulted in very little activity across the range of clinical isolates, as anticipated based on a lack of pharmacodynamic target attainment ($AUC/MIC \geq 125$ and $fT > MIC \geq 40\%$, respectively) for a majority of isolates tested (18, 19). Moreover, ceftazidime resulted in reliable efficacy for the isogenic OXA-48 strain and two of the clinical strains, particularly when MICs were $\leq 1 \mu\text{g/ml}$, corresponding to 100% $fT > MIC$. The preservation of good *in vitro* susceptibility and *in vivo* efficacy among these isolates is consistent with previously published *in vitro* hydrolysis data for the OXA-48 enzyme, demonstrating very little hydrolysis of extended-spectrum cephalosporins (3, 4). Much less efficacy was noted with ceftazidime against the four isolates with elevated MICs ($\geq 64 \mu\text{g/ml}$, 0% $fT > MIC$), secondary to the coproduction of extended-spectrum beta-lactamases (ESBLs). Nonetheless, the level of ceftazidime efficacy observed was fairly consistent with phenotypic and pharmacodynamic profiles across the isolates evaluated. These findings are also supported by two case reports in which susceptible OXA-48-producing *Enterobacteriaceae* were successfully treated with expanded-spectrum cephalosporins. A case of neonatal pneumonia caused by an OXA-48-producing *K. pneumoniae* isolate susceptible to ceftazidime and cefotaxime was successfully treated with a combination of cefotaxime and amikacin (20). Another patient with leukemia who developed a central line infection caused by an OXA-48-producing *Escherichia coli* strain, susceptible to all cephalosporins, successfully cleared their infection with a combination of ceftazidime and colistin (17). While ceftazidime may be a promising option for susceptible OXA-48 producers, there are still a large proportion of OXA-48-producing *Enterobacteriaceae* that coproduce other extended-spectrum beta-lactamases. A recent surveillance study that included 107 OXA-48-producing *Enterobacteriaceae* from European and North African countries found that 75% of these isolates coproduced ESBLs, with CTX-M-like enzymes being the most predominant, accounting for 89% of ESBL enzymes (21). Furthermore, 58.9% of these ESBL and OXA-48-coproducing isolates displayed *in vitro* resistance to ceftazidime. Consequently, ceftazidime may be a viable treatment option for OXA-48-producing *Enterobacteriaceae*, but only when *in vitro* susceptibility is demonstrated, as genotypic data are often not available in clinical settings.

High-dose, prolonged-infusion doripenem displayed some degree of efficacy against the isogenic and clinical strains tested in both models; however, the magnitude of bacterial reduction ap-

peared to be much less than that of the wild-type strain. Furthermore, the level of efficacy was not consistent across the entire range of isolates, despite having achieved the required pharmacodynamic target of $\geq 40\% fT > MIC$ (18). Our data demonstrating variable activity with this regimen appear to be consistent with prior *in vivo* and clinical observations. In a previous dose ranging study, ceftazidime demonstrated activity against a single susceptible OXA-48-producing strain, while imipenem lacked *in vivo* efficacy against this same isolate despite being imipenem susceptible. Furthermore, a number of case reports and case series describe treatment failures with imipenem- or meropenem-containing regimens when treating infections caused by OXA-48-producing *Enterobacteriaceae* (22–24). While the *in vitro* hydrolysis profile of doripenem with OXA-48 does not appear to have been evaluated to date, it is not entirely surprising that activity was still observed against some isolates evaluated in the current study, as there appears to be a difference in the level of hydrolysis among agents within the carbapenem class. In two separate enzyme kinetic studies of OXA-48, hydrolytic activity was much greater for imipenem than for meropenem and ertapenem in one study (3, 4). Since doripenem monotherapy was not as reliable as anticipated based on the phenotypic profile of the organisms included, future evaluations of carbapenem-based combination therapy regimens are warranted.

The *in vivo* activity observed with ceftazidime, levofloxacin, and ertapenem against the isogenic and clinical OXA-48-producing strains correlated well with pharmacodynamic targets and *in vitro* MIC. While levofloxacin and ertapenem did not result in efficacy against a majority of the strains evaluated as predicted based on elevated MICs, ceftazidime may represent a viable treatment option for OXA-48-producing *Enterobacteriaceae* when ceftazidime susceptible. Similar to previous *in vivo* and clinical observations of carbapenem failure against susceptible OXA-48-producing *Enterobacteriaceae*, activity with high-dose, prolonged-infusion doripenem was not consistent over the MIC range studied, despite this regimen achieving the requisite $fT > MIC$ needed for efficacy against all isolates. These data add to previous observations against KPC- and NDM-1-producing organisms suggesting that genotype may better predict carbapenem activity than the phenotypic profile for carbapenem-resistant *Enterobacteriaceae*.

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