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Evaluation of Ceftaroline Activity versus Daptomycin (DAP) against DAP-Nonsusceptible Methicillin-Resistant *Staphylococcus aureus* Strains in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model▽†

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The objective of this study was to investigate the potential role of ceftaroline, a new broad-spectrum cephalosporin, as a therapeutic option for the treatment of daptomycin-nonsusceptible (DNS) methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Four clinical DNS MRSA strains, R5717, R5563, R5996 (heteroresistant vancomycin-intermediate *S. aureus*) and R5995 (vancomycin-intermediate *S. aureus*) were evaluated in a two-compartment hollow-fiber *in vitro* pharmacokinetic/pharmacodynamic model at a starting inoculum of 10^7 CFU/ml for 96 h. Simulated regimens were ceftaroline at 600 mg every 12 h (q12h) (maximum free-drug concentration [fC_{max}], 15.2 μg/ml; serum half-life [$t_{1/2}$], 2.3 h), daptomycin at 6 mg/kg q24h (fC_{max}, 7.9 μg/ml; $t_{1/2}$, 8 h), and daptomycin at 10 mg/kg q24h (fC_{max}, 15.2 μg/ml; $t_{1/2}$, 8 h). Differences in CFU/ml between 24 and 96 h were evaluated by analysis of variance with Tukey's post-hoc test. Bactericidal activity was defined as a ≥3-$\log_{10}$ CFU/ml decrease in the colony count from the initial inoculum. The ceftaroline MIC values were 0.25, 0.5, 0.5, and 0.5 μg/ml, and the daptomycin MIC values were 2, 2, 4, and 4 μg/ml for R5717, R5563, R5996, and R5995, respectively. Ceftaroline displayed sustained bactericidal activity against 3 of the 4 strains at 96 h (R5717, −3.1 $\log_{10}$ CFU/ml; R5563, −2.5 $\log_{10}$ CFU/ml; R5996, −5.77 $\log_{10}$ CFU/ml; R5995, −6.38 $\log_{10}$ CFU/ml). Regrowth occurred during the daptomycin at 6-mg/kg q24h regimen (4 strains) and the daptomycin at 10-mg/kg q24h regimen (3 strains). At 96 h, ceftaroline was significantly more active, resulting in CFU/ml counts lower than those obtained with daptomycin at 6 mg/kg q24h (4 strains, $P \leq 0.008$) and daptomycin at 10 mg/kg q24 h (3 strains, $P \leq 0.001$). Isolates with increased MIC values for daptomycin (all 4 strains) but not for ceftaroline were recovered. Ceftaroline was effective against the 4 isolates tested and may provide a clinical option for the treatment of DNS MRSA infections.

Daptomycin (DAP)-nonsusceptible (DNS) *Staphylococcus aureus* is defined as a strain having an MIC of >1 μg/ml (5). In North America, the rate of DNS *S. aureus* is 0.01 to 0.1%, with no trend of increasing MIC values observed over the past several years (4, 27, 29, 33). DNS *S. aureus* infections occur mainly in patients with high-inoculum infections requiring long-term treatment such as osteomyelitis, medical devices, septic arthritis, or endocarditis (3, 35). Bactericidal therapies with a favorable safety profile are preferred to treat these types of infections. The recent guidelines from the Infectious Diseases Society of America for the treatment of methicillin-resistant *S. aureus* (MRSA) list telavancin, linezolid, trimethoprim-sulfamethoxazole, and quinupristin-dalfopristin as potential therapeutic options for the treatment of DNS *S. aureus* infections (19). Unfortunately, some of these agents and most other available alternative antistaphylococcal antibiotics, including tigecycline, clindamycin, and the tetracyclines, are typically bacteriostatic or may be limited by safety concerns. The optimal therapy for the treatment of infections caused by DNS *S. aureus* infections is therefore currently unknown (19, 35). Since the area under the concentration-time curve (AUC)/MIC or peak/MIC ratio is the pharmacodynamic parameter that best predicts efficacy, a "high dose" of DAP of 8 to 12 mg/kg/day has been evaluated clinically, showing both efficacy and a favorable safety profile (11, 22, 24). For this reason, high-dose DAP (10 mg/kg/day) is included in the recent MRSA guidelines to treat a variety of infections (19).

Ceftaroline (CPT), the active form of the prodrug CPT fosamil, is a novel broad-spectrum cephalosporin approved by the United States Food and Drug Administration in 2010 for the treatment of community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections. The current FDA-approved breakpoint for CPT against *S. aureus* (skin infections only) is ≤1 μg/ml (12). Similar to the mechanism of action of other cephalosporins, CPT binds to penicillin-binding proteins (PBPs), leading to inhibition of cell wall synthesis (17, 23, 40). CPT displays activity against many Gram-positive pathogens, including *Staphylococcus* spp. and *Streptococcus* spp. (10, 14, 15, 20, 25, 26, 30–32, 34). CPT has a strong affinity for PBP1, -2, and -3 and also maintains affinity for PBP2a, which is present in MRSA and confers resistance to methicillin and most other β-lactams (23, 40). In addition, CPT appears to maintain its susceptibility profile against strains of *S. aureus* displaying reduced susceptibility to vancomycin and DAP, including vancomycin-intermediate *S. aureus* (VISA), heteroresistant VISA (hVISA), vancomycin-resistant *S. au-*

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reus, and DNS *S. aureus*, as evidenced by MIC_{50} values of 0.5 μg/ml for DNS *S. aureus* (31, 34). CPT displays a favorable safety profile similar to those of other cephalosporins, with most adverse effects being mild (37, 41).

The objective of the present study was to investigate the potential of CPT as a therapeutic option for the treatment of DNS MRSA infections by using an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model.

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MATERIALS AND METHODS

Bacterial strains. A total of four clinical isolates of DNS MRSA obtained from patients with bacteremia at the Detroit Medical Center were evaluated, i.e., R5717, R5563, R5996 (hVISA), and R5995 (VISA). All isolates were tested by the previously described modified population analysis profile method using Mu3 as a control strain to determine hVISA or VISA status (42).

Antimicrobials and media. CPT (lot no. FMD-CEF-019 and FMD-CEF-028) was provided by its manufacturer (Forest Laboratories, Inc., New York, NY). CPT solutions were prepared daily in 50% dimethyl sulfoxide and 50% sterile water. DAP was commercially purchased. Mueller-Hinton broth II (MHBII; Difco, Detroit, MI) with 25 mg/liter calcium and 12.5 mg/liter magnesium was used for MIC testing and *in vitro* PK/PD models with CPT. MHBII supplemented to 50 mg/liter of calcium was used for MIC testing, and *in vitro* PK/PD models were used to evaluate DAP simulations due to the dependency of DAP on calcium for antimicrobial activity (18). Colony counts were determined using Tryptic Soy Agar (TSA; Difco, Detroit, MI) plates. Development of resistance was evaluated using either Mueller-Hinton agar (MHA; Difco, Detroit, MI) supplemented with 50 mg/liter of calcium or brain heart infusion agar (Difco, Detroit, MI) plates containing DAP and CPT, respectively.

Susceptibility testing. MIC values were determined by broth microdilution at 10^6 CFU/ml according to CLSI guidelines (5). All samples were incubated at 35°C for 24 h.

***In vitro* PK/PD model.** A previously described *in vitro* PK/PD model consisting of a two-compartment hollow-fiber model chamber (Fiber Cell Systems, Inc., Frederick, MD) with multiple ports for the addition and removal of growth medium, delivery of antibiotics, and collection of samples was used (39). Due to differences in the DAP penetration of various fiber model cartridges, a cellulosic fiber cartridge (C3008; Fiber Cell Systems, Inc., Frederick, MD) was utilized for all experiments with DAP and CPT. The apparatus was prefilled with medium, and antibiotics were administered as boluses over a 96-h time period. Prior to each experiment, several colonies from an overnight growth on TSA were suspended and added to each model to obtain a starting inoculum of ~10^7 CFU/ml. Fresh medium was continuously supplied to and removed from the central compartment along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL) set to simulate the human half-lives ($t_{1/2}$) of the antibiotics. The antibiotic regimens evaluated included CPT simulations of 600 mg every 12 h (q12h; maximum free-drug concentration [fC_{max}], 15.2 μg/ml; $t_{1/2}$, 2.3 h; protein binding, 20%) and DAP simulations of 6 mg/kg q24h (DAP6; fC_{max}, 7.9 μg/ml; $t_{1/2}$, 8 h; protein binding, 93%) and 10 mg/kg q24h (DAP10; fC_{max}, 13.17 μg/ml; $t_{1/2}$, 8 h; protein binding, 93%) (2, 13). All models were done in duplicate to ensure reproducibility.

Pharmacodynamic analysis. Samples from each model were collected at 0, 2, 4, 8, 24, 32, 48, 56, 72, and 96 h and diluted in cold 0.9% saline. Colony counts were determined by spiral plating appropriate dilutions using an automatic spiral plater (WASP; DW Scientific, West Yorkshire, England) to enumerate the CFU/ml. Colonies were counted using a laser colony counter (ProtoCOL; Synoptics Limited, Frederick, MD). If the drug concentration at the anticipated dilution was within 1 tube dilution of the MIC or higher, then vacuum filtration was used to avoid antibiotic carryover. When vacuum filtration was used, samples were washed through a 0.45-μm filter with normal saline to remove the antimicrobial agent and recover the bacteria on the filter, which was then placed on TSA plates. For both methods, plates were incubated at 37°C for 24 h, after which colony counts were performed. These methods have a lower limit of reliable detection of 1 $\log_{10}$ CFU/ml. The total reduction in $\log_{10}$ CFU/ml over 96 h was determined by plotting time-kill curves based on the number of remaining organisms. Bactericidal activity (99.9% killing) was defined as a ≥3-$\log_{10}$ CFU/ml decrease in the colony count from the initial inoculum. Bacteriostatic activity was defined as a <3-$\log_{10}$ CFU/ml reduction in the colony count from the initial inoculum, and inactivity was defined as no observed reduction of the initial inoculum. The time required to achieve a 99.9% bacterial load reduction was determined by linear regression or by visual inspection (if r^2 was ≤0.95). Antibiotic activity was assessed at every time point.

TABLE 1. MICs of tested isolates and recovered mutants[a]

Isolate and population[a]	MIC (μg/ml)		
	CPT[b]	DAP6	DAP10
R5717	0.25	2	2
T96	0.25	8	4–8
T96 M	NA[c]	8	8
R5563	0.5	2	2
T96	0.5	8	4
T96 M	NA	8	NA
R5995 (VISA)	0.5	4	4
T96	0.5	4	4
T96 M	NA	16	NA
R5996 (hVISA)	0.5	4	4
T96	0.5	8–16	4–8
T96 M	NA	8–16	16

[a] T96, overall bacterial population at 96 h; T96 M, mutant bacterial population at 96 h.
[b] CPT was used at 600 mg q12h.
[c] NA, no resistant mutants recovered.

Pharmacokinetic analysis. Pharmacokinetic samples were obtained through the injection port of each model at 0.5, 1, 2, 4, 8, 24, 32, 48, 56, 72, and 96 h in duplicate for the verification of target antibiotic concentrations. All samples were stored at −70°C until ready for analysis. CPT and DAP concentrations were determined by bioassay. For CPT, blank 0.25-in. disks were placed on preswabbed (*Bacillus subtilis* ATCC 6633) agar plates (antibiotic medium number 11) and spotted with 10 μl of the standards (2.5, 10, and 40 μg/ml) or samples. For DAP, 0.25-in. holes were punched in preswabbed (*Micrococcus luteus* ATCC 9341) agar plates (antibiotic medium number 5) and filled with 50 μl of the standards (2.5, 7.5, and 15 μg/ml) or samples. Each standard and sample was tested in duplicate. Plates were incubated for 18 to 24 h at 37°C, at which time the zone sizes were measured using a protocol reader (Protocol; Microbiology International, Frederick, MD). The $t_{1/2}$, fC_{max}, and fC_{min} of CPT and DAP were determined from the concentration-versus-time plots. The time above the MIC was calculated using first-order elimination concepts. The area under the concentration-time curve (AUC) and the subsequent AUC/MIC ratio were determined by the trapezoidal method utilizing PK Analyst Software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT).

Resistance. Development of resistance was evaluated at multiple time points throughout the simulation at 24, 48, 72, and 96 h. A sample (100 μl) from each time point was plated on MHA or brain heart infusion plates supplemented with 3 × MIC DAP or CPT to assess the development of resistance. Plates were then examined for growth after 24 and 48 h of incubation at 35°C.

Statistical analysis. Changes in the number of CFU/ml at 24, 48, 56, 72, and 96 h were compared by two-way analysis of variance with Tukey's post-hoc test. A P value of ≤0.05 was considered significant. All statistical analyses were performed using IBM SPSS Statistical Software (release 19.0; SPSS, Inc., Chicago, IL).

RESULTS

The MIC results for the isolates tested are summarized in Table 1. One isolate, R5717, displayed a CPT MIC value of 0.25 μg/ml, while the other three isolates displayed a MIC value of 0.5 μg/ml. The first two isolates, R5717 and R5563, were confirmed to be non-hVISA and non-VISA by population analysis and vancomycin susceptibility testing and displayed a DAP MIC value of 2 μg/ml. Isolate R5995, a VISA strain

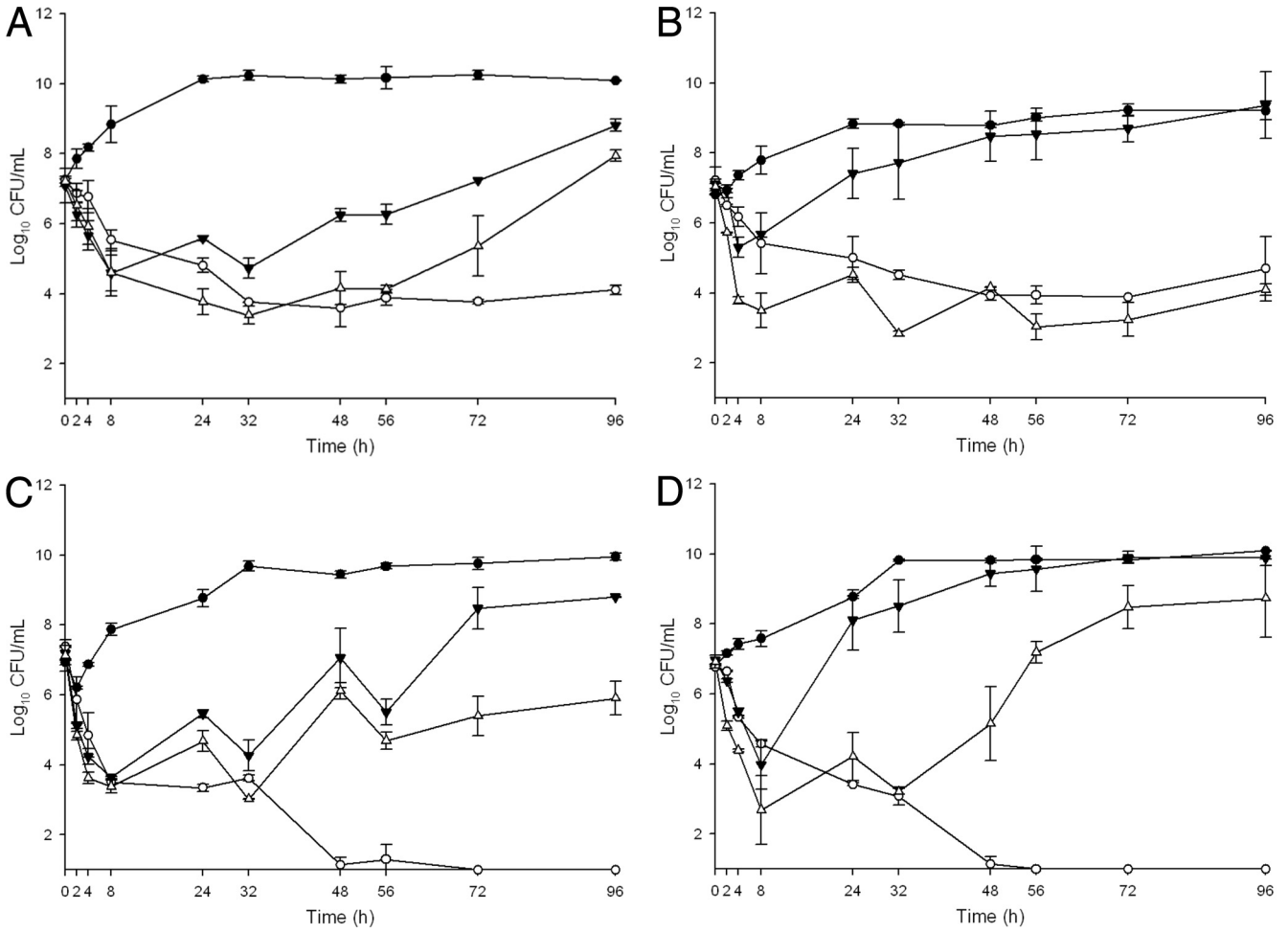

FIG. 1. Activities of CPT, DAP6, and DAP10 against R5717 (A), R5563 (B), R5995 (VISA) (C), and R5996 (hVISA) (D). Closed circles, growth control; open circles, CPT at 600 mg q12h; closed triangles, DAP6; open triangles, DAP10. Error bars are standard deviations of 2 independent experiments.

(ratio with Mu3, 1.23), and R5996, an hVISA strain (ratio with Mu3, 1.15), both displayed a DAP MIC value of 4 μg/ml.

The observed PK parameter values were within 12% of the target values for both drugs. For CPT, the observed fC_{max} values were 13.77 to 15 μg/ml (goal, 15.2 μg/ml) and the observed $t_{1/2}$ values were 2.25 to 2.47 h (goal, 2.3 h). The free time above the MIC ($fT>$MIC) was maintained for 100% of the 12-h dosing interval for R5717 and 95% (11.4 h) for the other three isolates. The observed fC_{max} and $t_{1/2}$ values were 8.65 to 8.84 μg/ml and 7.2 to 8.8 h for DAP6 (goal fC_{max}, 7.9 μg/ml; $t_{1/2}$, 8 h). The observed *f*AUC for DAP6 was approximately 108.7 μg-h/ml, corresponding to *f*AUC/MIC ratios of 54 and 27 for isolates with DAP MIC values of 2 and 4 μg/ml, respectively. DAP10 (goal fC_{max}, 13.17 μg/ml; $t_{1/2}$, 8 h) produced values of 13.59 μg/ml and 7.62 h for fC_{max} and $t_{1/2}$, respectively. The observed *f*AUC for DAP10 was approximately 149.4 μg-h/ml, corresponding to *f*AUC/MIC ratios of 75 and 37 for isolates with DAP MIC values of 2 and 4 μg/ml, respectively.

The quantitative changes in $\log_{10}$ CFU/ml are displayed in Fig. 1A to D. CPT displayed sustained bactericidal activity against 3 of the 4 strains, with overall decreases of 3.1 $\log_{10}$ CFU/ml (R5717), 2.5 $\log_{10}$ CFU/ml (R5563), 6.38 $\log_{10}$ CFU/ml (R5995), and 5.77 $\log_{10}$ CFU/ml (R5996) at 96 h. No mutants of any of the strains with elevated CPT MIC values were recovered, and the overall population MIC value did not change at 96 h (Table 1). The DAP6 regimen produced initial bactericidal activity against R5995 and R5996 and reductions in cell titers of 1.8 and 2.5 $\log_{10}$ for R5563 and R5717, respectively; however, this activity was not sustained and substantial regrowth of all four strains occurred. Under exposure to the DAP6 simulated regimen, the MIC values of the overall population increased from 2 to 8 μg/ml for R5717 and R5563 and from 4 to 8 to 16 μg/ml for R5996. Mutants of R5717/R5563 and R5995/R5996 displaying increased DAP MIC values of 8 and 16 μg/ml, respectively, were recovered. At 96 h, reductions in viable counts were significantly greater with CPT than DAP6 for all four strains ($P \leq 0.008$).

The simulated regimen of DAP10 displayed a dose-dependent increase in activity compared to DAP6. DAP10 was initially bactericidal against all four isolates. Regrowth of R5996 and R5717 occurred, with changes at 96 h of +1.8 and +0.7 $\log_{10}$ CFU/ml, respectively, versus the baseline. Mutants of both of these strains with DAP MIC values of 16 and 8 μg/ml,

respectively, were recovered. At 96 h, decreases in bacterial counts for the DAP10 regimen were 1.2 $\log_{10}$ CFU/ml for R5995 and 2.9 $\log_{10}$ CFU/ml for R5563. Mutants of neither of these strains were recovered during exposure to the DAP10 regimen. At 96 h, CPT resulted in reductions in viable counts of R5717 ($P = 0.001$), R5995 ($P < 0.001$), and R5996 ($P = 0.001$) significantly greater than those obtained with DAP10.

DISCUSSION

Although DNS *S. aureus* remains rare, when encountered as the cause of a clinical infection, it presents a treatment challenge, as optimal therapy is undefined (35). Recommended treatment options for infections caused by DNS *S. aureus* that also displays reduced susceptibility to vancomycin are limited to a small number of agents (if the strain is still susceptible) such as linezolid, quinupristin-dalfopristin, trimethoprim-sulfamethoxazole, and telavancin (19). Since DNS infections occur most commonly in patients with complicated deep-seated infections such as osteomyelitis, septic arthritis, and endocarditis, the optimal therapy to treat these infections would be an agent that is both bactericidal and relatively safe for longer treatment durations (3, 35).

In the present study, we examined the activity of CPT against four DNS MRSA strains, including an hVISA and a VISA strain. CPT displayed sustained bactericidal activity against three strains and achieved a sustained decrease in the bacterial counts of the fourth of 2.5 $\log_{10}$ CFU/ml, corresponding to a *fT*>MIC of 95 to 100%. During this experiment, no CPT-resistant mutants were recovered. In addition, CPT displayed enhanced activity with a greater than 5.5 $\log_{10}$ CFU/ml decrease in the colony counts of the two strains also displaying decreased susceptibility to vancomycin. This enhanced activity may be clinically relevant, because decreased DAP susceptibility has been noted in some VISA strains (9). In addition to its bactericidal activity, CPT appears to have a safety profile similar to that of other cephalosporins based on data from its clinical trials (6–8, 41). The combination of bactericidal activity and a favorable safety profile could make CPT a valuable potential option to treat MRSA infections, including those caused by DNS MRSA. We also observed variable activity of DAP at 6 and 10 mg/kg/day against the 4 DNS MRSA strains tested. DAP displayed a dose-dependent effect against all 4 strains tested. A DAP *f*AUC/MIC ratio of 56 to 222 is required for bactericidal activity (38). The *f*AUC/MICs achieved were in this range for DAP at 10 mg/kg versus R57175 and R5563 at 75, and sustained bactericidal activity was observed for R5563. In addition, exposure to DAP at 6 and 10 mg/kg/day produced mutants against 4 and 2 of the strains tested, respectively. The variable activity of high-dose DAP *in vitro* against DNS MRSA is consistent with previous reports from our laboratory (28).

Although the exact mechanisms of DNS in *S. aureus* have yet to be clearly elucidated, several changes in the cell membrane and surface have been associated with these strains. *S. aureus* strains with elevated DAP MIC values often display increased cell wall thickness, an increased cell membrane surface charge (causing repulsion of the DAP-Ca^{2+} complex), and changes in membrane fluidity (16, 21, 36). As CPT acts via the PBPs, including PBP2a in *S. aureus*, these phenotypic changes would not be expected to have a large effect, if any, on the activity of CPT (23, 40). The bactericidal activity and magnitude of the colony count decrease in this study are similar to those of a previously published study with a similar hollow-fiber model examining the activity of CPT against six *S. aureus* strains that were DAP susceptible, suggesting that the addition of DNS has little effect on CPT activity (39). Of these six strains, two also had decreased susceptibility to vancomycin with an hVISA phenotype. CPT displayed enhanced activity against one hVISA strain, R1629, killing to the limit of detection. For the other hVISA strain, regrowth occurred during exposure to the CPT regimen and this was explained by the higher CPT MIC value of 2 μg/ml and the heterogeneous population analysis profile.

Possible limitations of this study include its short duration, inoculum amounts, and the use of only one DNS strain each displaying the hVISA or VISA phenotype. As stated previously, most infections with DNS *S. aureus* require antibiotic treatment for long durations. It is therefore possible that the study period of 96 h (4 days) is not sufficient to elicit what the full antibiotic and DNS *S. aureus* interaction would be under longer exposures. The bacterial inoculum utilized for this study of 7 $\log_{10}$ CFU/ml is moderate compared to some infections typically associated with DNS *S. aureus* infections such as infective endocarditis. While the CPT MIC value range of strains utilized was 0.25 to 0.5 μg/ml, the CPT *fT*>MIC would be approximately 80% of the dosing interval for strains with a CPT MIC value of 1 μg/ml, easily exceeding the CPT *fT*>MIC target of ~45% for Gram-positive organisms (1). In this study, CPT displayed enhanced activity against the two DNS *S. aureus* strains also displaying an hVISA or VISA phenotype. Because there was only one strain of each type, the ability to extrapolate these results to other DNS *S. aureus* strains displaying decreased vancomycin susceptibility is limited.

In conclusion, CPT has the potential to provide a new therapeutic option for treating DNS MRSA infections, as it is a relatively well-tolerated antibiotic which could be more suitable for longer treatment durations while providing bactericidal activity. This is the first study evaluating the activity of CPT against DNS MRSA in an *in vitro* PK/PD model. CPT activity appeared to be enhanced for DNS *S. aureus* strains also displaying the hVISA or VISA phenotype. Further study of this enhanced activity and the efficacy of CPT for treating DNS *S. aureus* infections is warranted.

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