Daptomycin in Combination with Ceftolozane-Tazobactam or Cefazolin against Daptomycin-Susceptible and -Nonsusceptible *Staphylococcus aureus* in an *In Vitro*, Hollow-Fiber Model

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Ceftolozane-tazobactam (TOL-TAZ) is a novel cephalosporin/beta-lactamase inhibitor with activity against several Gram-negative pathogens. Daptomycin (DAP) has demonstrated synergistic activity with beta-lactams against methicillin-resistant *Staphylococcus aureus* (MRSA) isolates with reduced lipopeptide and glycopeptide susceptibilities. Our objective was to determine if DAP and TOL-TAZ possess synergy in hollow-fiber pharmacokinetic/pharmacodynamic (PK/PD) models. One isogenic pair of daptomycin-susceptible and daptomycin-nonsusceptible MRSA strains was evaluated. DAP, TOL-TAZ, and cefazolin (CFZ) MIC determinations were performed. DAP MIC determinations were also performed in the presence of subinhibitory concentrations of TOL-TAZ and CFZ. Ninety-six-hour *in vitro* models were run, simulating DAP at 10 mg/kg of body weight/day; TOL-TAZ at 1,500 mg every 8 h; TOL at 1,000 mg every 8 h; and DAP combined with TOL-TAZ (DAP+TOL-TAZ), DAP+TOL, DAP+TAZ, and DAP+CFZ at 2,000 mg every 8 h. DAP MICs were 0.5 and 4 µg/ml for strains R8845 and R8846, respectively. In the presence of CFZ, R8845 and R8846 DAP MICs were reduced 8-fold and 16-fold, respectively. TOL and TAZ had no effect on DAP MICs. PK/PD models demonstrated bactericidal activity with DAP+CFZ against both strains. The combination of DAP+TOL-TAZ was bactericidal against R8845 but was not bactericidal against daptomycin-nonsusceptible strain R8846. DAP+TOL and DAP+TAZ were not bactericidal. No other regimens were bactericidal. DAP+TOL-TAZ did not demonstrate synergistic activity against daptomycin-nonsusceptible *S. aureus* but prevented daptomycin-nonsusceptible MRSA emergence. Because DAP+TOL or TAZ alone did not prevent daptomycin-nonsusceptible MRSA emergence, the combination TOL-TAZ may be necessary for synergy with DAP. DAP+CFZ demonstrated enhancement against both strains. The combination of DAP+CFZ warrants further clinical study.

Staphylococcus aureus is the leading cause of hospital-associated infections in the United States, and methicillin-resistant *S. aureus* (MRSA) strains comprise up to 50% of isolates (1). More problematic is the emergence of MRSA isolates with reduced susceptibility to vancomycin, a drug which has been a mainstay of MRSA therapy for more than 40 years (2). Treatment options against such isolates are limited. Among them is daptomycin (DAP), a lipopeptide antibiotic with bactericidal activity against Gram-positive pathogens (3). Daptomycin maintains activity against MRSA isolates with reduced vancomycin susceptibility, and nonsusceptibility to daptomycin is rare, occurring in <0.05% of a sampling of >9,000 *S. aureus* isolates (4). Daptomycin nonsusceptibility has been reported, however, and creative therapies are needed to combat this problem (5–10). Recent data have demonstrated that beta-lactam antibiotics in combination with daptomycin are efficacious in both preventing daptomycin nonsusceptibility and providing bactericidal activity (6, 8, 10–13).

Because antibiotic resistance among Gram-negative bacteria is as much, if not more, of a problem as resistance among Gram-positive bacteria, several agents are under development or have recently been approved to combat Gram-negative infections. Ceftolozane (TOL) is a novel cephalosporin with activity against a broad range of Gram-negative pathogens, including excellent activity against *Pseudomonas aeruginosa* (14). When partnered with the beta-lactamase inhibitor tazobactam (TAZ), it possesses activity against expanded-spectrum-beta-lactamase-producing Gram-negative bacilli as well (14). The combination of ceftolozane and tazobactam has recently been approved for the treatment of com-

plicated intra-abdominal infections in combination with metronidazole as well as for the treatment of complicated urinary tract infections, including pyelonephritis (15, 16). Ceftolozane on its own lacks intrinsic Gram-positive activity, limiting its use against *S. aureus*. However, in critically ill patients, coinfection with several organisms is not uncommon, and coinfection by resistant *Pseudomonas aeruginosa* and MRSA isolates would provide a scenario in which ceftolozane may be administered concomitantly with daptomycin. Therefore, it is important to determine if exposure to ceftolozane-tazobactam enhances or antagonizes daptomycin activity. The goal of our study was to compare the activity of

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daptomycin alone to those of daptomycin in combination with ceftolozane alone, tazobactam alone, the combination of ceftolozane-tazobactam, or cefazolin (CFZ) in a pharmacokinetic/pharmacodynamic (PK/PD) model.

MATERIALS AND METHODS

Bacterial strains. One clinical isogenic pair of MRSA strains, one a daptomycin-susceptible strain (R8845) and the other a daptomycin-nonsusceptible strain (R8846), were selected for this study. These strains were chosen from our library at the Anti-Infective Research Laboratory.

Antimicrobials. Daptomycin was purchased commercially from Cubist Pharmaceuticals (Lexington, MA). Ceftolozane and tazobactam were obtained from Cubist Pharmaceuticals for the purpose of this study. Cefazolin was purchased commercially from Sigma Chemical Co. (St. Louis, MO).

Susceptibility testing and time-kill experiments. MIC values of the studied antimicrobials were determined in duplicate by broth microdilution at $\sim 10^6$ CFU/ml in Mueller-Hinton broth (MHB; Difco, Detroit, MI) supplemented with 50 μ g/ml calcium according to CLSI guidelines (17). Ceftolozane-tazobactam MICs were obtained at fixed concentrations of both 4 μ g/ml (CLSI standard) and 23.8 μ g/ml (maximum free-drug concentration in serum [fC_{max}]) of tazobactam to determine any possible effect of the tazobactam concentration on the ceftolozane MIC against these strains. Daptomycin MIC determinations were also performed in the presence of ceftolozane, tazobactam, ceftolozane-tazobactam, and cefazolin at $0.5\times$ the MIC of the respective organisms or the fC_{max} of the antibiotic, whichever value was lower, to determine the effects of these antimicrobials on daptomycin MIC reduction. Time-kill experiments were also performed against both strains with daptomycin, ceftolozane-tazobactam, cefazolin, and the combinations of daptomycin and ceftolozane-tazobactam and daptomycin and cefazolin. The initial bacterial inoculum was $\sim 10^6$ CFU/ml; daptomycin concentrations were $0.5\times$ MIC (0.25 μ g/ml for R8845 and 1 μ g/ml for R8846); ceftolozane and tazobactam concentrations represented the biological free peaks of 55 μ g/ml and 23.8 μ g/ml, respectively, against R8845 and $0.5\times$ MIC of ceftolozane (8 μ g/ml) with 23.8 μ g/ml tazobactam against R8846; and cefazolin concentrations were $0.5\times$ MIC (16 μ g/ml for R8845 and 1 μ g/ml for R8846). The ceftolozane concentration against R8846 was used based on the MIC obtained for ceftolozane of 16 μ g/ml in the presence of 23.8 μ g/ml tazobactam. Samples were collected at 0, 4, 8, 24, and 48 h, and colony counts ($\log_{10}$ CFU per milliliter) were compared among the regimens at 48 h. Synergy was defined as a combination regimen possessing a >100 -fold increase in bacterial killing compared to the most active single constituent, with one of the single constituents demonstrating no important effect on organism growth (18).

In vitro PK/PD model. An *in vitro*, hollow-fiber, pharmacokinetic/pharmacodynamic model with a 400-ml capacity and inflow and outflow ports was used. The target starting inoculum was $\sim 10^8$ CFU/ml. Fresh medium (MHB) was continuously supplied and removed from the compartment along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL) at an appropriate rate to simulate the average human clearance and half-lives ($t_{1/2}$) of the antimicrobials. The antimicrobial regimens evaluated were simulations of daptomycin at 10 mg/kg of body weight every 24 h (targeted fC_{max} of 11.3 μ g/ml, $t_{1/2}$ of 8 h, 92% protein binding, and free-drug area under the concentration-time curve [AUC] over 24 h [$fAUC_{0-24}$] of 114.8 μ g $\cdot$ h/ml) (19), ceftolozane-tazobactam at 1,500 mg every 8 h (targeted fC_{max} of 55 μ g/ml, $t_{1/2}$ of 2.7 h, and 20% protein binding for ceftolozane and targeted fC_{max} of 8 μ g/ml, $t_{1/2}$ of 2.7 h, and 30% protein binding for tazobactam) (20), daptomycin in combination with ceftolozane-tazobactam, daptomycin in combination with ceftolozane at 1,000 mg every 8 h without tazobactam, and daptomycin in combination with cefazolin at 2,000 mg every 8 h (targeted fC_{max} of 28 μ g/ml, $t_{1/2}$ of 2.7 h, and 93% protein binding) (21). A regimen of daptomycin in combination with tazobactam at 500 mg every 8 h was also run against R8845. The tazobactam pharma-

cokinetics were adjusted from an fC_{max} of 23.8 μ g/ml and a $t_{1/2}$ of 1 h to an fC_{max} of 8.8 μ g/ml and a $t_{1/2}$ of 2.7 h, with both regimens achieving identical AUC values. A regimen of daptomycin in combination with either ceftolozane or tazobactam alone was performed to determine if either component could be responsible for a potential synergistic effect with daptomycin. The models were performed in duplicate to ensure reproducibility. Supplemental daptomycin was added at an appropriate rate to ceftolozane, tazobactam, ceftolozane-tazobactam, and cefazolin to simulate the clearances of these beta-lactams (22).

Pharmacodynamic analysis. Samples from each model were collected at 0, 4, 8, 24, 32, 48, 72, and 96 h in duplicate. Colony counts were determined by spiral plating of appropriate dilutions using an automatic spiral plater (Wasp; DW Scientific, West Yorkshire, England). Colonies were counted by using a laser colony counter (ProtoCOL; Synoptics Limited, Frederick, MD). Bacteria were plated onto tryptic soy agar (TSA; Difco, Detroit, MI). These methods have a lower limit of reliable detection of $2 \log_{10}$ CFU/ml. The total reduction in $\log_{10}$ CFU per milliliter over 96 h was determined by plotting model time-kill curves based on the number of remaining organisms over the 96-h time period. Bactericidal activity was defined as a $\geq 3\text{-}\log_{10}$ CFU/ml (99.9%) decrease in colony counts from the initial inoculum. Bacteriostatic activity was defined as a $<3\text{-}\log_{10}$ CFU/ml reduction in colony counts from the initial inoculum. Enhancement of combinations was defined as a $\geq 2\text{-}\log_{10}$ CFU/ml reduction at 96 h for the combination compared to the colony counts with either agent alone.

Pharmacokinetic analysis. Ceftolozane and cefazolin concentrations were determined by a bioassay using *Escherichia coli* ATCC 25922. Each sample was tested in duplicate by placing antibiotic-permeated disks of known, standard antibiotic concentrations and disks permeated with unknown antibiotic concentrations from predetermined time points on agar plates (antibiotic medium number 11; Difco, Detroit, MI) inoculated with a suspension of the test organisms at a 0.5 McFarland standard. Ceftolozane and cefazolin MIC values for *E. coli* ATCC 25922 were determined in the presence of 11.3 μ g/ml daptomycin to ensure that there was no synergistic effect of daptomycin on the bioassay, and time-kill experiments were also performed with both agents in combination with daptomycin to further test for any synergistic activity (see the supplemental material). Daptomycin concentrations were determined by using standard high-performance liquid chromatography (HPLC) (23). The half-lives, AUC values, and peak concentrations of the antibiotics were determined by the trapezoidal method using PK Analyst software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT).

Resistance. Emergence of resistance was evaluated at 96 h by plating 100 μ l of samples from the models onto plates containing brain heart infusion agar (BHIA; Difco, Detroit, MI) supplemented with 50 mg/liter calcium and daptomycin at a concentration 3 times the daptomycin MIC for the tested organism. Resistant colonies growing on screening plates were evaluated by broth microdilution methods to determine the daptomycin MIC.

Statistical analysis. Changes in CFU per milliliter at 96 h were compared by one-way analysis of variance (ANOVA) for *in vitro* models. A *P* value of ≤ 0.05 was considered significant. All statistical analyses were performed by using SPSS statistical software (release 22; SPSS, Inc., Chicago, IL).

RESULTS

Susceptibility testing and time-kill experiments. MIC values of all antimicrobials against R8845 and R8846 are listed in Table 1. R8845 was susceptible to daptomycin, with an MIC of 0.5 μ g/ml, and R8846 was nonsusceptible, with an MIC of 4 μ g/ml. Daptomycin MIC values in the presence of the beta-lactams are also listed in Table 1. In the time-kill experiments with daptomycin, ceftolozane-tazobactam, and cefazolin against strain R8845, the combination of daptomycin and ceftolozane-tazobactam was bactericidal, synergistic, and statistically more effective at 24 h than the growth control, daptomycin alone, and ceftolozane-ta-

TABLE 1 MICs of antibiotics for tested organisms and DAP MICs in combination with TOL-TAZ, TOL, TAZ, or CFZ

Strain	MIC (μg/ml)								
	DAP	TOL-TAZ-4 ^a	TOL-TAZ-23.8 ^a	TOL	CFZ	DAP+TOL-TAZ-4 ^b	DAP+TOL ^b	DAP+TAZ ^b	DAP+CFZ ^b
R8845	0.5	128/4	128/23.28	128	32	0.25	0.5	0.5	0.06
R8846	4	32/4	32/23.8	32	2	4	4	4	0.25

^a Ceftolozane-tazobactam MICs were obtained with static tazobactam concentrations of both 4 μg/ml (TAZ-4) (CLSI standard) and 23.8 μg/ml (TAZ-23.8) (*fC_{max}* of tazobactam).
^b For combination MICs, TOL-TAZ, TOL, and CFZ were present in broth at 0.5× the reported MIC for each organism.

zobactam alone (*P* < 0.001) (Fig. 1). Daptomycin in combination with cefazolin was also bactericidal, synergistic, and statistically superior to each single-drug exposure (*P* < 0.001) (Fig. 1). The combinations of daptomycin plus cefazolin and daptomycin plus ceftolozane-tazobactam were statistically similar at 24 h.

In time-kill experiments against strain R8846, daptomycin alone, ceftolozane-tazobactam alone, and daptomycin in combination with ceftolozane-tazobactam displayed no appreciable activity at 24 h (Fig. 1). In contrast, daptomycin in combination with cefazolin was bactericidal, synergistic, and statistically superior to all other regimens tested (*P* < 0.001) (Fig. 1).

In vitro PK/PD models. The average observed *fC_{max}* for daptomycin was 10.8 μg/ml (target, 11.3 μg/ml), the average *fAUC_{0–24}* was 110.8 μg · h/ml (target, 114.8 μg · h/ml), and the average *t_{1/2}* was 7.7 h (target, 8 h) (19). The average observed *fC_{max}* for ceftolozane was 53.9 μg/ml (target, 55 μg/ml), and the average *t_{1/2}* was 2.5 h (target, 2.7 h) (20). The average observed *fC_{max}* for cefazolin was 29.3 μg/ml (target, 28 μg/ml), and the average *t_{1/2}* was 2.83 h (target, 2.7 h) (21). Time-kill and MIC data suggested that there was no synergistic activity between daptomycin and ceftolozane-tazobactam against reference *E. coli* strain ATCC 25922 (see the supplemental material).

Daptomycin alone was initially bactericidal against daptomycin-susceptible MRSA strain R8845 (Table 2 and Fig. 2A) but demonstrated regrowth at 32 h and did not maintain bactericidal activity at 96 h. At 96 h, the daptomycin MIC increased from 0.5 μg/ml to 2 μg/ml, representing a shift from daptomycin susceptibility to daptomycin nonsusceptibility. The *fAUC_{0–24}*/MIC ratio

of daptomycin for this organism based on the initial isolate MIC of the isolate was 221.6 μg · h/ml. Ceftolozane-tazobactam, ceftolozane alone, and cefazolin demonstrated little to no activity as single agents. Daptomycin in combination with ceftolozane-tazobactam (*fAUC_{0–24}*/MIC ratio based on an MIC of 443.2 μg · h/ml for daptomycin plus ceftolozane-tazobactam) was bactericidal at 96 h and demonstrated significantly greater activity than did daptomycin alone, ceftolozane-tazobactam alone, or daptomycin in combination with ceftolozane (*fAUC_{0–24}*/MIC ratio of 221.6 μg · h/ml) or tazobactam (*fAUC_{0–24}*/MIC ratio of 221.6 μg · h/ml) alone (*P* < 0.001). Daptomycin in combination with cefazolin (*fAUC_{0–24}*/MIC ratio of 1,772.8 μg · h/ml) was bactericidal by as soon as 4 h and remained bactericidal throughout the regimen, displaying statistically significantly more activity than any other regimen, including daptomycin plus ceftolozane-tazobactam (*P* = 0.025), at 96 h.

Daptomycin alone displayed little to no activity against daptomycin-nonsusceptible MRSA strain R8846 throughout the regimen (Table 2 and Fig. 2B). The *fAUC_{0–24}*/MIC ratio of daptomycin for this organism was 27.7 μg · h/ml. Daptomycin alone, ceftolozane-tazobactam alone, daptomycin in combination with ceftolozane-tazobactam (*fAUC_{0–24}*/MIC ratio of 27.7 μg · h/ml), and daptomycin in combination with ceftolozane (*fAUC_{0–24}*/MIC ratio of 27.7 μg · h/ml) were all statistically similar to the growth control at 96 h. However, the combination of daptomycin and cefazolin (*fAUC_{0–24}*/MIC ratio of 443.2 μg · h/ml) was bactericidal throughout the 96-h time period and statistically superior to all other regimens at the end of the model run (*P* < 0.001).

FIG 1 Twenty-four-hour time-kill experiments. The combination of daptomycin at 0.5× the MIC and ceftolozane-tazobactam at free biologic peaks was synergistic and bactericidal against R8845 at 48 h. DNS, daptomycin nonsusceptible.

TABLE 2 *In vitro* activities of regimens against R8845 and R8846 at 96 h

Regimen	Mean log ₁₀ CFU/ml at 96 h ± SD (mean change from baseline inoculum [log ₁₀ CFU/ml] ± SD) ^b	
	R8845	R8846
DAP	5.20 ± 0.14 (−2.60 ± 0.14)	10.60 ± 0.02 (+3.05 ± 0.07)
TOL-TAZ	9.40 ± 0.35 (+1.56 ± 0.33)	10.68 ± 0.08 (+2.84 ± 0.19)
DAP+TOL-TAZ	2.60 ± 0.14 (−5.29 ± 0.11)	10.19 ± 0.01 (+2.55 ± 0.13)
DAP+TOL	5.40 ± 0.14 (−2.40 ± 0.14)	9.95 ± 0.11 (+2.05 ± 0.11)
DAP+TAZ	5.33 ± 0.21 (−2.68 ± 0.23)	NA
DAP+CFZ	2.00 ± 0.00 (−6.05 ± 0.00) ^a	2.00 ± 0.00 (−6.10 ± 0.00) ^a
Growth control	10.75 ± 0.33 (+2.91 ± 0.05)	10.10 ± 0.38 (+2.37 ± 0.32)

^a The limit of detection for the CFU counting methods is 2 CFU/ml.^b NA, not applicable.

Cefazolin was the lone agent to increase the daptomycin $fAUC_{0-24}/MIC$ ratio against R8846 based on daptomycin MIC reduction.

DISCUSSION

Our study is the first to evaluate the novel cephalosporin ceftolozane in combination with daptomycin against resistant *S. aureus* isolates. Here, we have demonstrated that synergistic activity with daptomycin is not universal among all beta-lactam agents, as ceftolozane possessed little to no enhancement potential in these *in vitro* models. Interestingly, when tazobactam was paired with ceftolozane as in the clinical setting, enhancement of daptomycin activity against the daptomycin-susceptible strain was demonstrated. Converse to the combination of daptomycin and ceftolozane, the combination of daptomycin and cefazolin demonstrated bactericidal activity against both daptomycin-susceptible and daptomycin-nonsusceptible MRSA isolates.

The lack of synergistic activity between daptomycin and ceftolozane is disappointing if not altogether unexpected. Previously reported data demonstrated synergy between daptomycin and several beta-lactams against MRSA, but those beta-lactams all possessed at least some level of antistaphylococcal activity (6, 10–13, 24–27). Unlike those successful beta-lactams, ceftolozane lacks appreciable activity against Gram-positive organisms, which may be the reason for the lack of activity observed in our study. The

differences in synergistic activity may involve differential penicillin-binding protein (PBP) binding. One paper, in particular, suggested that beta-lactams with PBP1 activity, such as cefazolin and meropenem, seem to possess the best synergistic activity with daptomycin against *S. aureus* (28). To date, there are no data describing the PBP-binding profile of ceftolozane against Gram-positive bacteria, although a lack of activity against PBP1 may be a factor.

When ceftolozane and tazobactam were given together with daptomycin, the results were suggestive of synergistic activity, at least against the daptomycin-susceptible parent MRSA strain. Previously reported *in vitro* data demonstrate a synergistic relationship between daptomycin and piperacillin-tazobactam or ampicillin-sulbactam against MRSA (29). These data, combined with the lack of synergistic activity demonstrated in our study when either ceftolozane or tazobactam was given alone with daptomycin, suggest that the interaction between a beta-lactam and a beta-lactamase inhibitor may be capable of enhancing the activity of daptomycin against MRSA. The strain pair used in our study was part of a clinical case in which daptomycin at 10 mg/kg/day was unable to clear an infection, so the fact that daptomycin simulated at 10 mg/kg/day led to the development of daptomycin nonsusceptibility in our *in vitro* study is not surprising. Potentially, the mechanism of prevention of daptomycin nonsusceptibility may be related to the reduction of the R8845 daptomycin MIC in the presence of ceftolozane-tazobactam. This daptomycin MIC-lowering effect increased the daptomycin $fAUC_{0-24}/MIC$ ratio. Previous studies demonstrated that the daptomycin $fAUC_{0-24}/MIC$ ratio plays an important role in both preventing daptomycin nonsusceptibility and establishing bactericidal activity (30, 31). The increase in the daptomycin $fAUC_{0-24}/MIC$ ratio in the presence of ceftolozane-tazobactam may explain the lack of resistance development in strain R8845 that occurred when daptomycin was given alone. Although resistance prevention was established in our model, ceftolozane-tazobactam was not able to restore daptomycin activity against the already daptomycin-nonsusceptible strain R8846, suggesting that whatever effect that the combination of ceftolozane and tazobactam has on daptomycin activity does not extend to daptomycin-nonsusceptible strains. The combina-

FIG 2 Ninety-six-hour, hollow-fiber PK/PD models. Daptomycin (DAP) at 10 mg/kg/day in combination with cefazolin (CFZ) was bactericidal and demonstrated enhanced activity against both strains. The combination of DAP plus ceftolozane-tazobactam (TOL-TAZ) was bactericidal and demonstrated enhanced activity against only R8845. Activities of DAP+TOL and DAP+TAZ against R8845 were similar to those of DAP alone. (A) *S. aureus* R8845; (B) *S. aureus* R8846. GC, growth control; q24h, every 24 h.

tion of daptomycin with beta-lactam/beta-lactamase inhibitors is intriguing, and further study is warranted to determine if the synergistic relationship demonstrated here is more widely applicable.

The most striking observation from our study is the enhancement of daptomycin activity demonstrated by cefazolin. Cefazolin was chosen as a comparator beta-lactam due to its previously demonstrated synergy with daptomycin in time-kill data as well as its activity against Gram-positive organisms (28). Not only did cefazolin decrease the daptomycin MIC appreciably against both MRSA strains, it also demonstrated sustained bactericidal activity at 96 h in the PK/PD models when combined with daptomycin. Of interest, the cefazolin MIC decreased as the daptomycin MIC increased between the strains, demonstrating the presence of the “seesaw effect” phenomenon, where glycopeptide and lipopeptide MIC values are inversely proportional to those of beta-lactams (32, 33). Here, the seesaw effect was not present with ceftolozane-tazobactam, perhaps suggesting that antistaphylococcal beta-lactam activity may play an important role in daptomycin synergy or, perhaps, the demonstrated PBP1 binding of cefazolin. *In vitro* and clinical data previously demonstrated the efficacy of daptomycin in combination with antistaphylococcal beta-lactams against MRSA (6, 10–13, 26, 34, 35). Ceftaroline, in particular, has demonstrated the ability to enhance daptomycin activity against daptomycin-nonsusceptible strains of MRSA (6, 10, 11, 13, 26). However, there have been few reports regarding the efficacy of beta-lactams without anti-MRSA activity, e.g., cefazolin, in combination with daptomycin against daptomycin-nonsusceptible MRSA. Our data suggest that enhancement of daptomycin activity is not limited to beta-lactam agents with activity against the offending organism. The ability of cefazolin to enhance the activity of daptomycin has implications for therapy, as cefazolin does not possess the anti-MRSA activity of ceftaroline and may present a viable treatment strategy for prolonged outpatient therapy. Further study on this intriguing combination against daptomycin-nonsusceptible MRSA is warranted.

This study has some limitations. Only one strain pair was studied, so it is unclear if the effects demonstrated here are applicable to a broader range of isolates. Also, our models were run over only 96 h, which is a far shorter duration than what would be used clinically for most infections. The tazobactam pharmacokinetics were also different from those that are achieved clinically, although the peak was modified in conjunction with the half-life in order to achieve an $fAUC_{0-24}$ similar to that which is achieved clinically. In an effort to ensure that the extended tazobactam half-life had no effect on the models, a model was run by using the standard 1-h half-life of tazobactam in combination with daptomycin. These results were statistically similar to the values achieved in our model of daptomycin plus tazobactam with a 2.7-h half-life (see the supplemental material).

Conclusion. Daptomycin and ceftolozane-tazobactam represent important therapeutic options against resistant bacteria that are occurring with increasing frequency. The data from this study suggest that their use in combination does not present a risk for reduced efficacy against *S. aureus*. The combination of ceftolozane and tazobactam, possibly due to the tazobactam component, may even enhance the activity of daptomycin. Perhaps most importantly, we have demonstrated that cefazolin is efficacious in combination with daptomycin, even when MRSA isolates are daptomycin nonsusceptible. These *in vitro* observations provide valuable data that can be further evaluated with clinical use.

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