Impact of Dose De-Escalation and Escalation on Daptomycin's Pharmacodynamics against Clinical Methicillin-Resistant *Staphylococcus aureus* Isolates in an *In Vitro* Model[▽]

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De-escalation and escalation therapeutic strategies are commonly employed by clinicians on the basis of susceptibility results and patient response. Since no *in vitro* or *in vivo* data are currently available to support one strategy over the other for daptomycin, we attempted to evaluate the effects of dose escalation and de-escalation on daptomycin activity against methicillin-resistant *Staphylococcus aureus* (MRSA) isolates using an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model with simulated endocardial vegetations. Three clinical MRSA isolates, including one heterogeneous vancomycin-intermediate *S. aureus* (hVISA) isolate and one vancomycin-intermediate *S. aureus* (VISA) isolate, were exposed to daptomycin at 10 or 6 mg/kg of body weight/day for 8 days using a starting inoculum of $\sim 10^9$ CFU/g of vegetations, with dose escalation and de-escalation initiated on the fourth day. Daptomycin MIC values ranged from 0.5 to 1 μg/ml. In the PK/PD model, high-dose daptomycin (10 mg/kg/day) and de-escalation simulation (10 to 6 mg/kg/day) appeared to be the most efficient regimens against the three tested isolates, exhibiting the fastest bactericidal activity (4 to 8 h) compared to that of the standard regimen of 6 mg/kg/day and the escalation therapy of 6 to 10 mg/kg/day. The differences in the numbers of CFU/g observed between dose escalation and de-escalation were significant for the hVISA strain, with the de-escalation simulation exhibiting a better killing effect than the escalation simulation ($P < 0.024$). Although our results need to be carefully considered, the use of high-dose daptomycin up front demonstrated the most efficient activity against the tested isolates. Different therapeutic scenarios including isolates with higher MICs and prolonged drug exposures are warranted to better understand the outcomes of escalation and de-escalation strategies.

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have been increasingly reported in both the community and hospital settings and currently represent a serious health care threat (3, 30). Over the last decades, antibiotic overuse and misuse have largely contributed to the emergence of multidrug-resistant (MDR) pathogens by exerting a selective pressure on microorganisms present in the environment (24, 38). Independent of infection control measures, carefully considered antibiotic usage has proved to be highly beneficial in reducing the emergence of resistance (23, 33). Thus, implementation of antimicrobial stewardship programs internationally has been proposed to promote judicious use of antimicrobials and preserve the current anti-infective arsenal (2, 37). However, strategies developed to tackle the antibiotic resistance vary from country to country and within the different health care settings in the same country, highlighting the need for collective measures (5).

Daptomycin is one of the few options currently available to treat serious infections caused by MDR *S. aureus*, including isolates with reduced susceptibility to vancomycin (31, 41). The Food and Drug Administration approved the doses of 4 and 6 mg/kg of body weight/day over a 30-min intravenous infusion

for complicated skin and soft tissue infections (cSSSIs) and bloodstream infections, respectively (Cubicin package insert, Cubist Pharmaceuticals). However, despite the judicious therapeutic use of this agent in the past 7 years, isolates of MRSA with reduced susceptibility to daptomycin have emerged. In a recent and extensive review of the literature, Falagas et al. reported seven daptomycin-nonsusceptible isolates over 60 clinical cases of endocarditis and bacteremia (13), including four MRSA strains recovered from patients who previously received vancomycin and three vancomycin-resistant enterococci (13). Although the rate of resistance to daptomycin is a significantly lower than that to most antimicrobials, it appears to be essential to optimize the use of this drug and limit the risk of emergence of nonsusceptible organisms (32). Clinicians often start daptomycin therapy using the approved dosage of 6 mg/kg/day and then increase the dose if the patient's clinical and/or microbiological response is not adequate (19, 20, 29). Depending on the site of infection and the ability of the antimicrobial to reach the site in adequate concentrations, the strategy of dose escalation may be comparable to the gradient exposure method commonly used in laboratory-based science to encourage the development of resistance *in vitro* (6). On the other hand, daptomycin has been proved to be a potent concentration-dependent bactericidal agent, and use of high-dose daptomycin initially may limit the development of resistance through its more rapid bactericidal activity (7, 34). Postmarketing observational studies evaluating the efficacy and safety of high-dose daptomycin in difficult-to-treat *S. aureus* infec-

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tions have reported promising results (29). As an alternative to clinical investigations, we report in this study on the effect of dose escalation and de-escalation on daptomycin's *in vitro* activity against three clinical isolates of MRSA, including one vancomycin-intermediate *S. aureus* (VISA) strain and one heterogeneous vancomycin-intermediate *S. aureus* (hVISA) strain.

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MATERIALS AND METHODS

Bacterial strains. A total of three clinical MRSA were selected from the Anti-Infective Research Laboratory MRSA collection. Isolates included one vancomycin- and daptomycin-susceptible MRSA isolate (B010-01, recovered from a patient at the Ohio State University Medical Center in 2009), one hVISA isolate (R3099, recovered from the bloodstream of a patient of the Detroit Medical Center in 2005), and one VISA isolate (NRS-118, from the Network on Antimicrobial Resistance in *Staphylococcus aureus* collection). The hVISA characteristic of R3099 was confirmed by use of a modified population analysis profile and Mu3 as a reference strain, as previously described by Wootton et al. (data not shown) (42) The VISA phenotype of NRS-118 was confirmed by macro-Etest, as described elsewhere (25).

Antimicrobials. Daptomycin analytical powder (Cubist Pharmaceuticals, Inc., Lexington, MA) was provided by its manufacturer, whereas vancomycin was commercially purchased from Sigma-Aldrich (St. Louis, MO). Drug stock solutions were freshly prepared every day according to the CLSI guidelines (10) or the manufacturer's recommendations.

Media. Due to daptomycin's dependence on calcium for antimicrobial activity, Mueller-Hinton (MH) broth (Difco, Detroit, MI) supplemented with 50 or 75 μg/ml calcium and 12.5 μg/ml magnesium (50 and 75 SMHB, respectively) was used for all susceptibility testing and *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) models evaluating daptomycin, respectively (22). Tryptic soy agar (TSA; Difco, Detroit, MI) and Mueller-Hinton agar (Difco, Detroit, MI) plates were used for colony counting and detection of emergence of resistance, respectively.

Susceptibility testing. MICs of daptomycin and vancomycin were determined in duplicate by broth microdilution at ~5.5×10^5 CFU/ml, as recommended by the CLSI guidelines (10).

Pharmacokinetic/pharmacodynamic models. The previously described *in vitro* PK/PD model with simulated endocardial vegetations (SEVs) was used to evaluate the effects of de-escalation and escalation regimens on daptomycin's killing activity against three clinical isolates of *S. aureus* (27). Briefly, the central chamber of the apparatus containing 32 SEVs was prefilled with 75 SMHB and maintained at 37°C in a water bath. SEVs were prepared as previously described by mixing the organism suspension, human cryoprecipitate from volunteer donors (American Red Cross, Detroit, MI), platelets, and bovine thrombin. This mixture resulted in simulated vegetations containing a starting bacterial burden of 10^9 CFU/g with approximately 3 to 3.5 g/dl of albumin and 6.8 to 7.4 g/dl of total protein (1). Daptomycin was administered as daily boluses over a 192-h period via an injection port. A magnetic stir bar was placed in the central compartment to ensure mixing of the drug throughout the procedure. Fresh medium was continuously supplied and removed from the compartment along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL) set to simulate the half-life of daptomycin (targeted at 8 h) (4) A total of four regimens were evaluated in duplicate for each isolate to ensure reproducibility and included daptomycin simulations of 10 mg/kg/day (peak concentration, 129.7 μg/ml) (4) for 4 days followed by 6 mg/kg/day (peak concentration, 95.7 μg/ml) (4) for 4 days, daptomycin simulations of 6 mg/kg/day for 4 days followed by 10 mg/kg/day for 4 days, daptomycin simulations of 10 mg/kg/day for 8 days, and daptomycin simulations of 6 mg/kg/day for 8 days. Total drug concentrations were used, since all experiments were performed in the presence of a concentration of albumin simulated to match that in humans. A growth control was performed for each isolate to ensure viability of the organisms through the experiment.

Pharmacodynamic analysis. Two simulated endocardial vegetations (total of four) were removed at 0, 4, 8, 24, 32, 48, 56, and 72 h for days 0 to 3 and 0, 4, 8, 24, 32, 48, 56, 72, and 96 h for days 4 to 8. The SEVs were homogenized, diluted in cold normal saline, and plated onto TSA plates to allow colony counting. When serial dilution did not prevent antibiotic carryover, samples were vacuum

TABLE 1. *In vitro* activity of daptomycin against 3 isolates of MRSA

Isolate no. (DAP MIC [μg/ml])	Regimen[a]	Change in $\log_{10}$ CFU/g from starting inoculum at:	
		96 h	192 h
B010-01 (1)	D6	-5.31 ± 0.25	-3.62 ± 0.61
	D6-10	-4.68 ± 0.47	-8.20 ± 0.00
	D10	-8.57 ± 0.22	-8.70 ± 0.00
	D10-6	-8.18 ± 0.00	-8.18 ± 0.00
R3099 (1)	D6	-5.10 ± 0.35	-4.96 ± 0.04
	D6-10	-5.35 ± 0.21	-4.88 ± 0.42
	D10	-6.75 ± 0.12	-5.87 ± 0.42
	D10-6	-6.87 ± 0.09	-5.59 ± 0.13
VISA NRS-118 (0.5)	D6	-4.89 ± 0.39	-5.18 ± 0.56
	D6-10	-3.94 ± 0.61	-5.02 ± 0.27
	D10	-7.16 ± 0.09	-7.55
	D10-6	-6.85 ± 0.09	-6.44 ± 0.14

[a] D6, daptomycin at 6 mg/kg/day for 8 days; D6-10, daptomycin at 6 mg/kg/day for 4 days followed by 10 mg/kg/day for 4 days; D10, daptomycin at 10 mg/kg/day for 8 days; D10-6, daptomycin at 10 mg/kg/day for 4 days followed by 6 mg/kg/day for 4 days.

filtered through a 0.45-μm-pore-size filter before they were plated, therefore reducing the antibiotic concentration below the MIC of the drug. We determined these methods to have a lower limit of reliable detection of 1 $\log_{10}$ CFU/g. Plates were incubated at 35°C for 24 h, at which time colony counts were performed. The total reduction in $\log_{10}$ CFU/g over 192 h was determined by plotting time-kill curves on the basis of the number of remaining organisms over the time period. Bactericidal activity (99.9% kill) and bacteriostatic activity were defined as ≥3-$\log_{10}$ CFU/g and <3-$\log_{10}$ CFU/g reductions in the colony count from the initial inoculum, respectively. Inactivity was defined as no observed reductions from the initial inoculum. The time required to achieve a 99.9% bacterial load reduction was determined by linear regression (if r^2 was ≥0.95) or visual inspection.

Pharmacokinetic analysis. Samples for pharmacokinetic analysis were obtained through the injection port at 0.5, 1, 2, 4, 8, 24, 32, 48, 56, and 72 for days 0 to 3 and 4 to 8 for verification of target antibiotic concentrations. All samples were stored at −70°C until they were ready for analysis. Concentrations of daptomycin were determined by microbioassay utilizing *Micrococcus luteus* ATCC 9341, as previously described (35). This assay demonstrated a lower limit of detection of 5 μg/ml and interday and intraday coefficients of variation less than or equal to 10% for 50-, 100-, and 200-μg/ml standards. The half-life, area under the curve (AUC), and peak (maximum) concentration (C_{max}) 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 throughout the simulation at multiple time points every 24 h. Briefly, at each time point 100-μl samples were plated on MH agar plates supplemented with calcium (final concentration, 50 mg/liter) and containing 3× MIC of daptomycin. Plates were examined for growth after 24 and 48 h of incubation at 35°C. Any growth observed was tested for changes in susceptibility by both Etest and the microdilution method according to the CLSI recommendations (10).

Statistical analysis. Changes in the numbers of CFU/g between regimens at 24, 48, and 72 h for days 1 to 3 and 96, 120, 144, 168, and 192 h for days 4 to 8 were compared by two-way analysis of variance with Tukey's *post hoc* test using SPSS statistical software (release 18.0; SPSS, Inc., Chicago, IL). A *P* value of ≤0.05 was considered significant.

RESULTS

Daptomycin MIC values were 0.5, 1, and 1 μg/ml for VISA NRS-118, B010-01, and hVISA R3099, respectively. Except for VISA NRS-118 (MIC = 4 μg/ml), the vancomycin MIC of all isolates was 2 μg/ml (Table 1). No change in daptomycin MIC

was observed throughout the 8-day period of daptomycin exposure for any of the 3 isolates tested.

Pharmacokinetic analysis demonstrated the accuracy of the models performed, with C_{max} and half-life values being within 10% of the targeted values. The total peak concentrations observed were 127.37 ± 2.1 and 96.4 ± 2.3 µg/ml for daptomycin regimens of 10 and 6 mg/kg/day, respectively, and the daptomycin half-life was approximately 7.35 ± 0.33 h. In terms of pharmacodynamic activity, daptomycin demonstrated a concentration-dependent effect and sustained bactericidal activity, although some variability between strains was observed over the 8 days of drug exposure (Fig. 1). Depending on the regimen used to start the simulations, daptomycin achieved 99.9% of the killing between 4 and 32 h, with the highest dose of 10 mg/kg/day exhibiting the most rapid cidal effect (between 4 and 8 h). Against B010-01, the use of daptomycin at 10 mg/kg/day up front or applied 4 days after the dose of 6 mg/kg/day held the bacterial population down significantly, reaching the limit of detection at 56 h (Fig. 1A). In contrast, the use of a regimen of 6 mg/kg/day for 8 days significantly reduced the killing activity of daptomycin, with a 4-$\log_{10}$-CFU/g bacterial regrowth observed at 152 h. No change in MIC was observed at this point, but the population analysis profile revealed a shift toward the highest MIC within the population following a standard dose of 6 mg/kg/day for 8 days (Fig. 2, dashed lines). In contrast, escalation to a dose of 10 mg/kg/day at 96 h after 4 days with a dose of 6 mg/kg/day resulted in a significant killing effect, with the bacterial burden achieving the limit of detection at 152 h and no colonies being detected at 192 h (Fig. 1A). Against hVISA isolate R3099, daptomycin regimens of 10 mg/kg/day and 10 to 6 mg/kg/day resulted in more effective killing effects than the regimens of 6 mg/kg/day and 6 to 10 mg/kg/day (Fig. 1B). Except at 128 and 192 h, the differences between escalation and de-escalation were statistically significant ($P <$ 0.02), with the de-escalation regimen resulting in greater activity than the escalation regimen. Analysis of the initial population MICs revealed a heterogeneous profile for susceptibility to daptomycin and the presence of subpopulations with higher MICs, which may explain the reduced activity of 10 mg/kg/day for the hVISA isolate than the MRSA strain (Fig. 2, open squares). Finally, against VISA isolate NRS-118, the killing effect observed with the dose de-escalation regimen appeared to be slightly greater than the activity resulting from the escalation simulation (Fig. 1C). Except at 100 and 120 h, the difference between the two regimens was not statistically significant ($P >$ 0.05), but bactericidal activity was achieved much more rapidly with the de-escalation regimen or daptomycin at 10 mg/kg/day up front. Similar to the findings for hVISA strain R3099, no increase in MIC was observed at the end of the experiment, and the heterogeneous profile of the initial population might explain the reduced activity of daptomycin and the small differences between the regimens evaluated (Fig. 2, open diamonds).

DISCUSSION

It is well-recognized that the PK and PD parameters of a drug play a key role in the success or failure of a therapy as well as in the emergence or the selection of resistant subpopulations (16, 40). In this study, we aimed to investigate how spe-
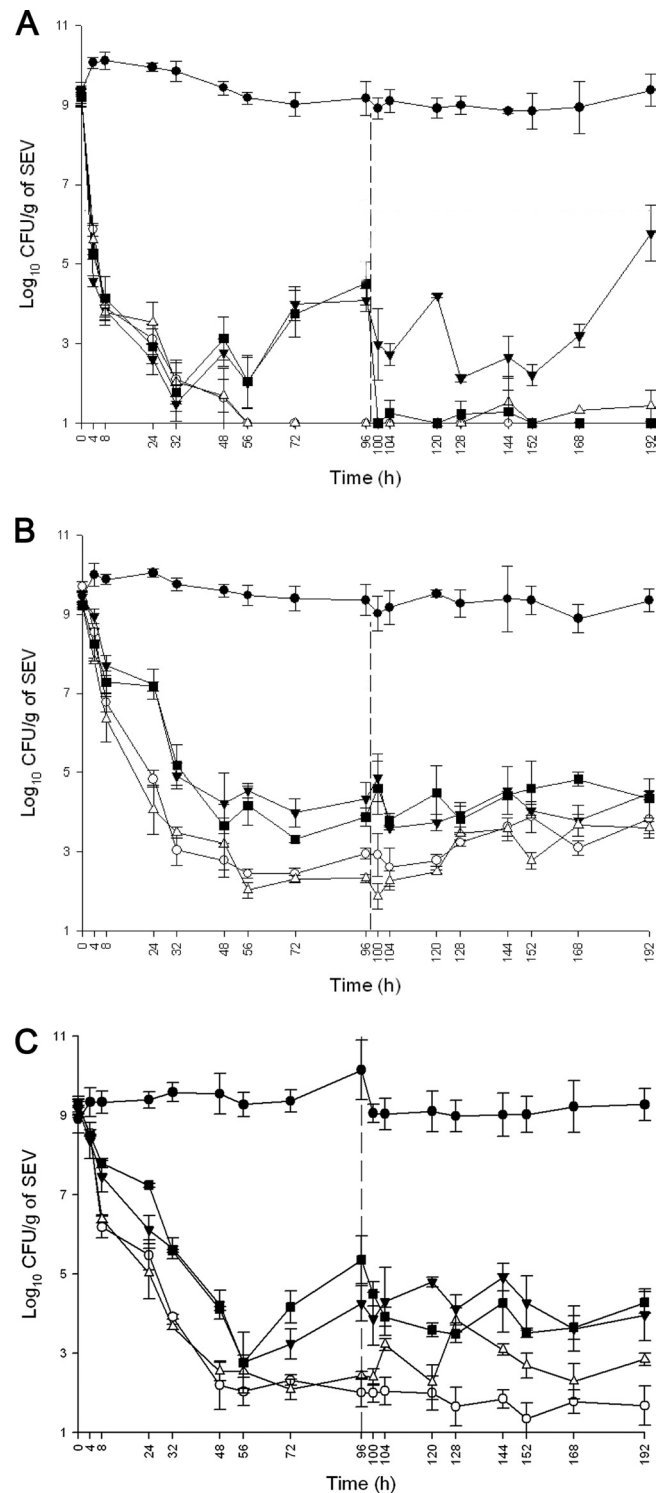

FIG. 1. *In vitro* activity of daptomycin. (A) B010-01; (B) hVISA R3099; (C) VISA NRS-118. Filled circles, growth control; open circles, daptomycin at 10 mg/kg/day; open triangles, daptomycin at 10 to 6 mg/kg/day; filled inverted triangles, daptomycin at 6 mg/kg/day; filled squares, daptomycin at 6 to 10 mg/kg/day. The vertical dashed line represents the time for de-escalation or escalation therapy, if it was performed.
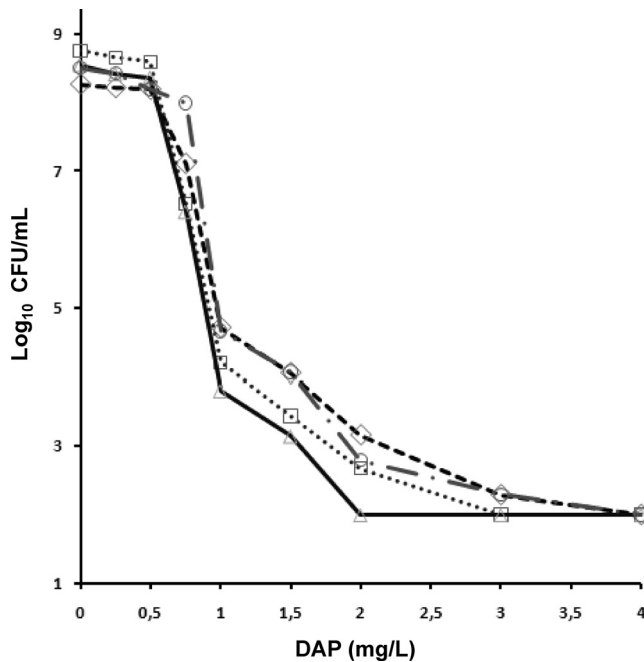
FIG. 2. Daptomycin population analysis profiles. Open triangles, B010-01 at 0 h; open circles, B010-01 at 192 h; open squares, R3099 at 0 h, open diamonds, NRS-118 at 0 h.

cific PK changes as a result of escalation or de-escalation of the dose regimens would affect the killing activity of daptomycin against isolates of MRSA in the context of a simulated high inoculum burden, such as those found in infective endocarditis. Consistent with previously published data, we observed daptomycin concentration-dependent bactericidal activity against all MRSA isolates, including strains with reduced susceptibility to vancomycin (26, 34). Also consistent with previous results from our group (26), in comparison with the results for the standard dose (6 mg/kg/day), high-dose daptomycin (10 mg/kg/day) achieved a more rapid and the greatest killing effect against all isolates, reaching the limit of detection (i.e., less than 10 CFU/g of vegetation) at 56 h for one isolate. Despite some limitations, including evaluation of a small number of isolates and a short length of therapy (8 days *in vitro* versus at least 4 weeks *in vivo*) (15), our data tend to support the use of high-dose daptomycin up front with a potential for use of a de-escalation dosage.

Bactericidal antimicrobials are usually preferred to bacteriostatic antibiotics for the treatment of infective endocarditis. The reasons behind this rationale include the limited host defenses at the site of infection as well as the need for rapid bacterial eradication to shorten the therapy and reduce the risk of resistance, relapse, or complications (12). The importance of rapid bacterial clearance has recently been explored by Chang et al. in a prospective observational study that included 505 patients with *S. aureus* bacteremia (9). Patients were monitored for 6 months and up to 3 years in order to identify any relapse or reinfection episodes secondary to vancomycin or nafcillin therapy. In all patients with methicillin-susceptible *S. aureus* bacteremia, nafcillin therapy appeared to be superior to vancomycin therapy with respect to persistent bacteremia

and/or relapse prevention. Infective endocarditis was reported to be one of the risk factors for relapse of *S. aureus* bacteremia, and a multivariate analysis of treatment demonstrated that vancomycin was an independent predictor of relapse ($P = 0.05$ or 6.5; 95% confidence interval, 1.0 to 52.8). Chang et al. concluded that a more rapid clearance of bacteremia is associated with a lower likelihood of complications (9). These results were recently confirmed by Khatib et al. (18) and highlight the importance of rapid bacterial eradication in the treatment of complicated bacteremia, including infective endocarditis. The temporal relationship between antibiotic therapy and the occurrence of complications secondary to infective endocarditis has not yet been clearly investigated. However, recent data report that patients (about 40%) with left-sided endocarditis may experienced cerebrovascular complications in the first 10 days following the initiation of appropriate antibiotic therapy and before sterilization of the endocardial vegetations (9, 14, 15). Such complications can still occur during the first week, despite adequate treatment. However, the fact that the risk of complication is rapidly decreased after initiation of the administration of antibiotics supports a faster sterilization of the vegetation and clearance of the bacteremia to reduce the risk of embolization and relapse (12).

Reports of daptomycin-nonsusceptible strains of MRSA in the past few years, especially in patients with bacterial endocarditis, emphasize the need to optimize therapy to achieve clinical success (17, 21, 39, 43). The mechanism responsible for the reduced susceptibility of these isolates to daptomycin is not completely understood. Increased expression of specific loci involved in membrane thickness, composition, and charges, such as *dltABCD* (43) and *mprF* (21), has been suggested. Previous exposure to vancomycin leading to reduced bactericidal *in vitro* activity of daptomycin (11, 32) has also been reported, with the subsequent emergence of daptomycin-nonsusceptible isolates secondary to daptomycin exposure (39). We recently reported on the superiority of a dose of 10 mg/kg/day over one of 6 mg/kg/day using an *in vitro* model of SEVs, with the development of reduced susceptibility to daptomycin occurring using a dose of 6 mg/kg/day, whereas a dose of 10 mg/kg/day prevented the emergence of such isolates (36). Similar results were reported with a clinical daptomycin-nonsusceptible strain selected *in vivo* with a dose of 6 mg/kg/day, and *in vitro* time-kill assays performed with 8 μg/ml (equal to a free concentration of a 6-mg/kg/day dose regimen) showed that bactericidal activity against this isolate was retained (39). Finally, in a rabbit model of endocarditis, Chambers et al. reported on the potential for higher efficacy from a 10-mg/kg/day dose, irrespective of the daptomycin susceptibility profile of the strain (8). Taken together, these data suggest that high-dose daptomycin may prevent the selection or development of isolates with reduced susceptibility to daptomycin and therefore subsequent clinical failure. Our data also suggest that the use of de-escalation from high-dose daptomycin to 6 mg/kg/day and continuous high-dose daptomycin at 10 mg/kg/day demonstrated similar results. This may indicate that sustained use of 10 mg/kg/day throughout the treatment may not be necessary and may not offer an advantage from the standpoint of improved safety.

Although clinicians may be more comfortable with the practice of escalating dosages when patients do not respond initially, the use of off-label high-dose daptomycin (i.e., 10 mg/

kg/day) up front may present more challenges. Therefore, clinicians will need to weigh the risks and benefits of this dosing procedure. Observational data from high-dose daptomycin use would suggest that it may be safe; however, there are no large-scale clinical trials at this point to confirm this initial information, so caution is warranted.

In conclusion, because we observed a more rapid reduction of the bacterial burden in the SEVs with high-dose daptomycin (10 mg/kg/day) applied continuously or de-escalated to 6 mg/kg/day, this strategy may lead to a faster cure of bacteremia *in vivo* and to prevention of the emergence of reduced susceptibility to daptomycin. The findings in the increasing inventory of reports recently published also suggest the potential for the efficacy and safety of high-dose daptomycin and support the conclusions of the present study (4, 28). Use of a de-escalation strategy could eventually be considered a reasonable alternative, but *in vivo* investigations are warranted to determine the appropriate length of high-dose daptomycin that would ensure the sterilization of the vegetations, preventing further infectious embolus complications and the development of nonsusceptible strains.

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