In Vitro Pharmacodynamics of Human Simulated Exposures of Telavancin against Methicillin-Susceptible and -Resistant *Staphylococcus aureus* with and without Prior Vancomycin Exposure

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Telavancin is a lipoglycopeptide with potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA). The activity of telavancin against MRSA and MSSA after prior vancomycin exposure was studied in an *in vitro* pharmacodynamic model. Two clinical MRSA and two MSSA isolates, all with vancomycin MICs of 2 µg/ml, were subjected to humanized free drug exposures of vancomycin at 1 g every 12 h (q12h) for 96 h, telavancin at 750 mg q24h for 96 h, and vancomycin at 1 g q12h for 72 h followed by telavancin at 750 mg q24h for 48 h (120 h total). The microbiological responses were measured by changes from 0 h in log₁₀ CFU/ml at the end of experiments and area under the bacterial killing and regrowth curves over 96 h (AUBC_{0–96}). The control isolates grew to 8.8 ± 0.3 log₁₀ CFU/ml. Initially, all regimens caused –4.5 ± 0.9 reductions in log₁₀ CFU/ml by 48 h followed by slight regrowth over the following 48 to 72 h. After 96 h, vancomycin and telavancin achieved –3.7 ± 0.9 and –3.8 ± 0.8 log₁₀ CFU/ml changes from baseline, respectively (*P* = 0.74). Sequential exposure to telavancin after vancomycin did not result in additional CFU reductions or increases, with ultimate log₁₀ CFU/ml reductions of –4.3 ± 1.1 at 96 h and –4.2 ± 1.3 at 120 h (*P* > 0.05 for all comparisons at 96 h). The AUBC_{0–96} was significantly smaller for the regimen of telavancin for 96 h than for the regimens of vancomycin for 96 h and vancomycin followed by telavancin (*P* ≤ 0.04). No resistance was observed throughout the experiment. Against these MRSA and MSSA isolates with vancomycin MICs of 2 µg/ml, telavancin was comparable with vancomycin and its activity was unaffected by prior vancomycin exposure.

Staphylococcus aureus is one of the most globally prevalent infectious organisms in both hospital and community settings (1). Methicillin-resistant *S. aureus* (MRSA), in particular, represents a health care threat with >80,000 cases of invasive infections estimated to occur annually in the United States and 11,000 deaths according to a recent report by the Centers for Disease Control and Prevention (2). MRSA is a common cause of bloodstream infections, skin infections, and pneumonia (3). In addition to the risk of mortality, infections due to MRSA are also linked to an increased length of hospital stay and increased hospitalization costs (4).

For decades, intravenous vancomycin has been considered the standard of care for treatment of MRSA infections in hospitalized patients and is often empirically initiated in patients when *S. aureus* is suspected. However, vancomycin therapy may fail in some patients due to increases in the MIC up to 2 µg/ml (i.e., MIC creep) or secondary to the development of nephrotoxicity, which is reported to occur at a rate of 4 to 42% (5, 6). Therefore, alternative anti-MRSA agents are needed for patients who do not respond to vancomycin.

Only in the recent decade has the pharmaceutical industry caught up with treatment options for resistant *S. aureus* infections. Several intravenous antibiotics that harbor activity against MRSA are now available. Typically, these newer antibiotics are reserved for serious infections, such as pneumonia and bacteremia, after failure of vancomycin therapy. Therefore, it would be important to understand if prior exposure to vancomycin might influence the activity of the newer agents. Previous *in vitro* studies have identified the potential for prior vancomycin exposure to result in the development of daptomycin heteroresistance (7, 8); however, a similar effect on ceftaroline was not observed.

Telavancin is a lipoglycopeptide with potent *in vitro* activity against Gram-positive bacteria, including MRSA, methicillin-susceptible *S. aureus* (MSSA), heteroresistant vancomycin-intermediate *S. aureus* (hVISA) (vancomycin MIC of ≤2 µg/ml and a ratio of a population analysis profile to the area under the bacterial growth curve [PAP/AUC] of ≥0.9 compared with that for the reference isolate Mu3), and VISA (vancomycin MIC of 4 to 8 µg/ml) (9, 10, 11). Telavancin is approved for the treatment of complicated skin and skin structure infections, as well as hospital-acquired and ventilator-associated bacterial pneumonia due to MRSA and MSSA, and is currently being studied for complicated bacteremia caused by *S. aureus*. Given the glycopeptide core structure of telavancin, its mechanism of action resembles, in part, that of vancomycin, although a second telavancin mechanism of action is present that affects the bacterial cell membrane through the disruption of its barrier function (11). Like many new anti-MRSA antibiotics, telavancin is expected to be reserved for serious infections in patients who have not responded to vancomycin. Little is known about the effect of prior vancomycin exposure on the telavancin efficacy against MRSA and MSSA.

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MATERIALS AND METHODS

Bacterial strains and susceptibility testing. A collection of clinical MRSA and MSSA isolates from skin, respiratory tract, and blood sources were screened for inclusion in the study. Isolates (2 MRSA and 2 MSSA) were selected based on a vancomycin MIC of 2 µg/ml to simulate the worst-case scenario for a vancomycin-susceptible organism. MICs were determined in triplicate via broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) guidance (12). Isolates also had to be susceptible to telavancin (MIC of ≤0.125 µg/ml) and daptomycin (MIC of <1 µg/ml) and not harbor any vancomycin heteroresistance (as described below). *S. aureus* ATCC 29213 was used as an internal quality control strain. Polysorbate 80 (Tween 80; Sigma-Aldrich, St. Louis, MO) was added to the broth used in the MIC testing of telavancin according to the manufacturer's recommendation (11). Calcium chloride dihydrate (Sigma-Aldrich, St. Louis, MO) was added to the broth used in the MIC testing of daptomycin (13).

Determination of vancomycin heteroresistance. To exclude hVISA isolates, a modified population analysis profile (mPAP) was conducted on the candidate isolates using a previously described method (14, 15). Briefly, an inoculum of approximately 10⁸ CFU/ml density of each isolate in addition to the reference hVISA isolate, Mu3 (ATCC 700689), was prepared on brain heart infusion (BHI) agar plates containing 0, 0.5, 1, 1.5, 2, 3, 4, 8, and 16 µg/ml of vancomycin. The ratio of the area under the bacterial growth curve of the *S. aureus* isolates tested to the corresponding area of Mu3 was calculated. A ratio of ≥0.9 was required to consider an isolate hVISA.

Antibiotics. Telavancin standard powder (lot 70621AA076) was provided by Theravance Biopharma Antibiotics, Inc. (George Town, Cayman Islands). Stock solutions were prepared with the addition of polysorbate 80 (Tween 80) at a concentration of 0.002%, according to the manufacturer's recommendations (11). Vancomycin (lot 27400DD, expiration March 2015; Hospira, Inc., Lake Forest, IL) was obtained from the Department of Pharmacy at Hartford Hospital in Hartford, CT, and was used in the *in vitro* pharmacodynamic (PD) experiments. Vancomycin (lot WXBB5169V, expiration March 2016; Sigma-Aldrich, St. Louis, MO) and daptomycin (lot 010903A, expiration March 2015; Cubist, Inc., Lexington, MA) standard powders were utilized for the MIC testing.

Simulated drug exposures. Humanized free drug exposures of vancomycin at 1 g every 12 h (q12h) were simulated based on the mean parameter estimates from a population pharmacokinetic model where the target area under the free drug concentration-time curve from 0 to 12 h ($fAUC_{0-12}$) for vancomycin was 105 µg · h/ml based on targeting a free peak concentration of 14 µg/ml, a free trough concentration of 5 µg/ml, and a half-life of 8.2 h (16). Likewise, telavancin exposure was simulated at a dose of 750 mg (10 mg/kg, assuming a 75-kg patient) q24h based on the mean parameter estimates from the population pharmacokinetic model published by Samara and colleagues (17) where the target $fAUC_{0-24}$ was 80.1 µg · h/ml and was obtained through targeting a free peak of 10.7 µg/ml, a free trough of 1.25 µg/ml, and a half-life of 1.1 h for the first hour (to simulate the distribution phase) and 9.9 h from 1 to 24 h (to simulate the terminal elimination phase). The protein binding rates of vancomycin and telavancin were assumed to be 50% and 90%, respectively, when free drug exposures were simulated (17, 18). All simulations were performed on ADAPT 5 (USC Biomedical Simulations Resource, Los Angeles, CA). Three treatment regimens were employed during the study. Each was used in a separate experiment against the two selected MRSA and MSSA strains: (i) single-drug therapy with vancomycin for 96 h, (ii) single-drug therapy with telavancin for 96 h, and (iii) single-drug therapy with vancomycin for 72 h followed sequentially, 12 h after the last dose of vanco-

mycin, by single-drug therapy with telavancin for 48 h (i.e., 120 h total experiment duration).

In vitro pharmacodynamic model. A one-compartment *in vitro* chemostat pharmacodynamic model was used as previously described (19). Briefly, three independent, 1,000-ml glass chemostat models were implemented in each experiment (two experimental treatment models and one growth control model). All three models were run simultaneously for each isolate and were performed in duplicate to ensure reproducibility. All models were filled with 1,000 ml of cation-adjusted Mueller-Hinton II broth (MHB) (Becton, Dickinson and Company, Sparks, MD) supplemented with polysorbate 80 (Tween 80) to achieve a final concentration of 0.002% of the latter, as recommended for telavancin MIC testing in broth. So that all organisms were growing in the same medium, the same method was followed for control and vancomycin experiments. The models were placed in water baths with controlled temperature at 35°C. Magnetic stir bars were used for adequate mixing of the model contents.

A starting inoculum of 10⁸ CFU/ml was used to simulate a higher inoculum in an invasive infection. Briefly, the inoculum was prepared after transfer of each isolate twice on a Trypticase soy agar plate with 5% sheep blood (TSA II) (Becton, Dickinson and Company, Sparks, MD). On the day of the experiment, the TSA II plates were washed with normal saline using a sterile spreader until a uniform bacterial suspension was made, which was then transferred to a 50-ml tube that was filled with normal saline to 40 ml. This was split in half, and the tubes were filled to 42 ml with normal saline. The extra 2 ml was used for bacterial density confirmation. The tubes containing 40 ml of the suspension were centrifuged at 2,500 rpm at 25°C for 20 min. After the supernatant was poured off, 3 ml of normal saline was added to each tube, and the contents were mixed and poured into one tube to serve as the bacterial suspension pool. Ten milliliters of the bacterial suspension was injected into each of the three models and allowed to enter the log growth phase for 30 min before antibiotic dosing was started.

Antibiotics were added as boluses into the treatment models to achieve the target peak concentrations. Fresh broth was supplied via a peristaltic pump (Masterflex L/S model 7524-40; Cole-Parmer Instrument Company, Vernon Hills, IL) set to achieve the human simulated half-life of the antimicrobial being tested. Samples were obtained from each of the models at specific time points throughout the experiment and were serially diluted in normal saline to assess changes in bacterial density over time. Aliquots from each diluted sample were plated onto TSA II plates and incubated at 37°C for 18 to 24 h for quantitative cultures. The time-kill curves were constructed by plotting the log₁₀ CFU/ml against time. The lower limit of quantification was 1.7 log₁₀ CFU/ml.

Antibiotic concentration determination. Samples from all treatment models were collected during each dosing interval to confirm the achievement of target exposures. Upon collection, samples were immediately frozen and stored at -80°C until analyzed. Vancomycin concentrations were determined using a previously validated high-performance liquid chromatography (HPLC) assay at the Center for Anti-Infective Research and Development (Hartford, CT) (20). Telavancin samples were analyzed by Theravance Biopharma US, Inc. (South San Francisco, CA). A protein precipitation extraction was utilized to prepare samples for injection onto a high-performance liquid chromatography-tandem mass spectrometer (HPLC/MS-MS) system. A stable label isotope of telavancin was used as an internal standard, and standard curves were prepared in broth matrix.

Resistance determination. To assess for the potential emergence of resistance, telavancin, vancomycin, and daptomycin MICs of the isolates tested were determined in triplicate using broth microdilution at 48 and 96 h for the single-drug experiments and at 72 and 120 h for the sequential drug experiments. Bacterial inocula were prepared directly from the TSA II plates containing model samples and were not retransferred prior to MIC testing to avoid conversion of any heteroresistance development back to the wild-type phenotype.

Statistics. The primary endpoint was the comparative change in log₁₀ CFU/ml from 0 h at the end of the experiment (96 h or 120 h) between

TABLE 1 MICs of MRSA and MSSA strains selected for use in the *in vitro* pharmacodynamic model^a

Isolate	MIC (μg/ml)		
	Telavancin	Vancomycin	Daptomycin
MRSA 360	0.063	2	0.5
MRSA F1-3	0.063	2	0.5
MSSA F4-24	0.125	2	0.5
MSSA F9-28	0.125	2	0.5

^a Vancomycin-intermediate *S. aureus* heteroresistance (hVISA) was ruled out in all isolates by the mPAP method.

vancomycin, telavancin, and sequential treatment regimens. The secondary endpoint was the assessment of the area under the bacterial killing and regrowth curves (AUBC) at 96 h for single-drug and sequential experiments. These endpoints were compared by analysis of variance using the Holm-Sidak method for multiple comparisons. The rates of killing of MRSA and MSSA by vancomycin were compared to those of telavancin by calculating the slopes of the killing curves and comparing the values using Student's *t* test. An *a priori* *P* value of ≤ 0.5 was used to determine the statistical significance. Data were analyzed using SigmaPlot, version 12.5 (Systat Software, Inc., San Jose, CA).

RESULTS

Isolates. Two MRSA strains (MRSA 360 and F1-3) and two MSSA strains (MSSA F4-24 and F9-28) were selected for the *in vitro* experiments. The MICs are provided in Table 1. hVISA was ruled out in all selected isolates.

Pharmacokinetic analysis. The targeted and observed exposures of each treatment are shown in Table 2. The observed free drug concentrations, *f*AUC, and half-life for telavancin were within 11% of the target. Vancomycin had an observed peak and trough that were 23% and 30% above the target, respectively. However, both the *f*AUC and the half-life of vancomycin were within 20% of the target.

Antibacterial results. The mean starting inocula of the MRSA and MSSA isolates were similar at $8.4 \pm 0.2 \log_{10}$ CFU/ml. The control isolates of MRSA and MSSA grew to 8.9 ± 0.1 and $8.7 \pm 0.2 \log_{10}$ CFU/ml, respectively. The time-kill curves of all of the treatment regimens against each isolate are shown in Fig. 1A to D. Initially, all regimens resulted in -4.5 ± 0.9 reductions in $\log_{10}$ CFU/ml by 48 h, followed by slow regrowth over the course of the following 48 h. Over the initial 24 h, telavancin resulted in faster killing of the MSSA isolates than vancomycin (mean slopes of -0.016 and -0.013 for telavancin and vancomycin, respectively; $P = 0.01$); no differences were observed for the MRSA isolates (mean slopes of -0.011 and -0.014 for telavancin and vancomycin, respectively; $P = 0.12$). After 96 h, vancomycin and telavancin treatments achieved -3.7 ± 0.9 and $-3.8 \pm 0.8 \log_{10}$ CFU/ml changes from the baseline inocula, respectively ($P = 0.74$). The sequential expo-

sure to telavancin after 72 h of prior vancomycin exposure did not result in additional CFU reductions or increases, with ultimate $\log_{10}$ CFU/ml reductions of -4.3 ± 1.1 at 96 h and -4.2 ± 1.3 at 120 h ($P > 0.05$ for all comparisons with vancomycin or telavancin) (Table 3). All treatment regimens achieved significant CFU reductions compared with those for the control models ($P < 0.001$). The AUBC results over the first 96 h are provided in Table 4. The AUBC of the telavancin single-drug regimen for 96 h was significantly lower than those for vancomycin for 96 h and the sequential drug regimen ($P = 0.04$ and 0.03 , respectively). The results were similar when analyzed for each isolate and when the MRSA and MSSA phenotypes were combined.

Resistance. All four MRSA and MSSA isolates retained their initial MICs against telavancin, vancomycin, and daptomycin at all of the MIC testing time points; thus, no development of resistance was observed.

DISCUSSION

Telavancin has often been used for serious MRSA infections after poor responses to vancomycin or other antibiotics (21, 22, 23, 24). The effect of prior vancomycin exposure on the activity of telavancin against *S. aureus* is, however, not well understood. In this experiment, we used the *in vitro* pharmacodynamic model to evaluate human-simulated free drug concentrations of telavancin after exposure to 72 h of vancomycin at 1 g q12h.

Against the 4 isolates tested, we saw no further increase in killing, but also no negative (i.e., regrowth) effect when telavancin was simulated after 72 h of vancomycin exposure. This observation was in contrast to observations seen with daptomycin and ceftaroline against MRSA, hVISA, and VISA in a study by Bhalodi and colleagues after 48 h of vancomycin exposure (8). In that study, bacterial growth ensued upon exposure to daptomycin following vancomycin exposure secondary to the development of daptomycin heteroresistance. In contrast, ceftaroline, a β -lactam antibiotic, continued to kill the bacteria after vancomycin exposure with no development of resistance. In the current study, telavancin appears to fall somewhere in between, as portrayed by a plateau-like appearance of the killing curves (Fig. 1A to D). Potential explanations include the possibility that the simulated vancomycin exposure reached the threshold of bacterial killing in the model, and thus no further killing ensued upon exposure to telavancin. Alternatively, bacterial tolerance to glycopeptides may have developed, since telavancin has a structure and mechanism of action similar to those of vancomycin. The potential development of tolerance may have blunted the bactericidal effect of telavancin; however, the development of vancomycin tolerance was not assessed in our studies to confirm this rationale. Interestingly, a study by Rose and colleagues showed that telavancin

TABLE 2 Targeted and observed pharmacokinetics of vancomycin and telavancin against all isolates

Antibiotic regimen	<i>f</i> AUC (μg · h/ml) ^a		<i>f</i> Peak (μg/ml)		<i>f</i> Trough (μg/ml)		<i>t</i> _{1/2} (h) ^b	
	Target	Observed	Target	Observed	Target	Observed	Target	Observed
Vancomycin (1 g q12h)	105	126.3 ± 20.3	14	17.2 ± 3	5	6.5 ± 0.9	8.2	8.4 ± 0.9
Telavancin (750 mg q24h)	80.1	84.9 ± 9.6	10.7	10.1 ± 1.8	1.3	1.2 ± 0.5	9.9	8.8 ± 1.6

^a *f*AUC of the dosing interval for vancomycin (12 h) and telavancin (24 h).

^b The half-life (*t*_{1/2}) of telavancin is based on the duration from 1 h to 24 h. The target half-life for the first hour after telavancin exposure was 1.3 h (observed, 1.9 ± 0.3 h).

FIG 1 Mean bacterial densities over 96 h for single-drug treatment regimens and over 120 h for sequential regimens by isolate. (A) MRSA 360; (B) MRSA F1-3; (C) MSSA F4-24; (D) MSSA F9-28. ●, growth controls; ■, vancomycin for 96 h; ▲, telavancin for 96 h; ◇, vancomycin for 72 h followed by telavancin for 48 h.

maintained its antibacterial activity against MRSA isolates that were exquisitely vancomycin tolerant (25).

Our study showed killing profiles that were largely similar for telavancin and vancomycin against MRSA and MSSA isolates, thereby supporting the noninferiority of the two agents seen in clinical trials (26, 27). Notably, in the current study, telavancin demonstrated a killing pattern that was faster than

that of vancomycin in the first 24 h against the MSSA isolates only. In contrast, more rapid killing was shown with MRSA in previous studies when telavancin was compared with other anti-MRSA drugs (28, 29, 30). Secondary to the faster killing pattern demonstrated by telavancin and lower CFU counts between 48 to 72 h (Fig. 1A to D), the AUBC for telavancin for 96 h was significantly lower than that of vancomycin for 96 h and

TABLE 3 Mean observed changes in log₁₀ CFU/ml for each treatment regimen against the isolates tested

Regimen ^a	$\Delta\log_{10}$ CFU/ml ^b						
	MRSA 360	MRSA F1-3	MSSA F4-24	MSSA F9-28	MRSA (n = 2)	MSSA (n = 2)	All isolates ^c
VAN for 96 h ^c	-4.1 ± 0.6	-3.5 ± 1.6	-3.4 ± 0.2	-4.0 ± 0.6	-3.7 ± 1.3	-3.8 ± 0.6	-3.7 ± 0.9
TLV for 96 h ^c	-4.2 ± 0.4	-4.8 ± 0.2	-2.9 ± 0.4	-3.6 ± 0.2	-4.5 ± 0.4	-3.3 ± 0.5	-3.9 ± 0.8
VAN for 72 h-TLV for 48 h							
Results at 96 h	-5.0 ± 2.2	-4.4 ± 1.3	-3.3 ± 0.2	-4.3 ± 0.4	-4.7 ± 1.5	-3.8 ± 0.6	-4.3 ± 1.1
Results at 120 h	-5.2 ± 2.1	-3.5 ± 1.3	-3.2 ± 0.6	-4.9 ± 0.1	-4.4 ± 1.7	-4.1 ± 1.1	-4.2 ± 1.3

^a VAN, vancomycin; TLV, telavancin.

^b Data are presented as the means ± SD of all observations.

^c Based on data from all isolates, no statistically significant difference was noted between vancomycin and telavancin ($P = 0.7$) or between either therapy with sequential therapy at either 96 h or 120 h ($P > 0.05$ for all comparisons). Against MRSA and MSSA, telavancin was not different from vancomycin ($P = 0.4$ and 0.3 , respectively), and neither was different from the sequential regimen at 96 h or 120 h ($P > 0.05$ for all comparisons). All regimens achieved statistically significant reductions compared with those for the controls against MRSA, MSSA, and all isolates collectively ($P < 0.01$).

TABLE 4 Mean area under the bacterial killing and regrowth curves for each treatment regimen against the isolates tested^a

Regimen	AUBC ^b		
	MRSA	MSSA	All isolates ^c
Vancomycin ^d	492.1 ± 64.3	515.3 ± 39.8	503.7 ± 52.4
Telavancin ^d	436.3 ± 8	461.8 ± 49.7	450.9 ± 38
Vancomycin-telavancin ^d	475.7 ± 49.5	555.2 ± 60.4	515.5 ± 66.5

^a Data are presented as the means ± SD from two models during each experiment.^b AUBC, area under the bacterial killing and regrowth curve.^c Based on the data from all isolates, a statistically significant difference was noted between vancomycin and telavancin ($P = 0.04$) and between telavancin and sequential therapy ($P = 0.03$). However, no difference between vancomycin and sequential therapy was observed ($P = 0.6$). Against MRSA and MSSA, the AUBC of telavancin was not different from that of vancomycin ($P = 0.2$ and 0.1 , respectively), and neither was different from that of the sequential regimen at 96 h ($P > 0.05$ for both comparisons) except the telavancin versus sequential regimen against MSSA, where the AUBC of the former was lower than that of the latter ($P = 0.02$). Compared with the control models, the AUBC of all treatment regimens were significantly lower than the AUBC of the control models against MRSA, MSSA, and all isolates collectively ($P < 0.001$).^d Results at 96 h.

the sequential regimen. This suggests that telavancin might display an advantage earlier in therapy with respect to bacterial load reductions. Despite this observation, however, the CFU count at 96 h did not differ between telavancin and vancomycin, and both agents were no different from the sequential therapy in terms of ultimate CFU count.

Multiple studies have compared the *in vitro* pharmacodynamics of telavancin with vancomycin against *S. aureus* with different resistance phenotypic profiles. A study by Smith and colleagues (29) demonstrated a sustained bactericidal activity of telavancin versus comparators, including vancomycin. However, the investigators did not consider a biphasic elimination during their telavancin exposures, so the telavancin AUC exposures ($\sim 122 \mu\text{g} \cdot \text{h/ml}$) were higher than what is typically observed in humans. Similarly, telavancin was superior to vancomycin against MSSA, MRSA, and hVISA in previous studies (31, 32).

In our studies, MRSA and MSSA isolates did not develop resistance to vancomycin, telavancin, or daptomycin after exposure to vancomycin or telavancin for up to 96 to 120 h. Taglietti and colleagues (33) observed an increase in cell wall thickness with a subsequent increase in the vancomycin MIC from $\leq 0.5 \mu\text{g/ml}$ to $2 \mu\text{g/ml}$ after serial passaging of 19 MRSA isolates on vancomycin-supplemented agar; these isolates also displayed an increase in the telavancin MIC to $\leq 1 \mu\text{g/ml}$, but all remained susceptible based on the telavancin breakpoint at the time. Notably, the MIC testing method and the susceptibility breakpoint ($\leq 0.125 \mu\text{g/ml}$) have changed due to the use of polysorbate 80 to prevent adherence of the drug to plastic surfaces and minimize MIC variability (11, 34). Nonetheless, we utilized isolates with baseline vancomycin MICs of $2 \mu\text{g/ml}$, which may already have variable mechanisms for lower susceptibility to vancomycin, including a thickened cell wall; thus, no further increases in the MICs may be feasible with the exposures simulated in the experiment. The emergence of resistance to telavancin was shown, however, in an *in vitro* dose range-finding study, but only when the $f\text{AUC}/\text{MIC}$ ratio was in the range of 1 to 10 (using the older MIC method), and no resistance development was observed when the $f\text{AUC}/\text{MIC}$ ratio was > 400 (35). This may explain the lack of resistance devel-

opment in our studies since the calculated $f\text{AUC}/\text{MIC}$ ratios were 1,348 and 679 for the tested MRSA and MSSA isolates, respectively.

Potential limitations to the study exist, which are important to recognize when the data from this *in vitro* pharmacodynamic model are interpreted. First, *in vitro* experiments do not account for the human immune system which may aid the bactericidal effect of antibiotics, thus simulating the worst-case scenario. Second, exposure to vancomycin in these experiments (i.e., $f\text{AUC}_{0-12}$ of $126 \pm 20 \mu\text{g} \cdot \text{h/ml}$) was 20% higher than the targeted exposure. However, the observed AUC value falls within the range of vancomycin exposures seen in clinical practice, where $f\text{AUC}_{0-12}$ after a vancomycin dose of 1,000 mg was reported in several population pharmacokinetic studies of vancomycin to range from 116 to $172 \mu\text{g} \cdot \text{h/ml}$ (36, 37, 38). Another limitation was the selected durations of 96 to 120 h since infections due to *S. aureus* are often treated for periods longer than 7 days; hence, a longer exposure may have elicited the full impact of vancomycin and telavancin therapy, as well as increased the probability of resistance development over time. However, the simulation of vancomycin for the 72 h prior to the switch to telavancin may mimic the actual clinical practice where the early clinical response within the first 3 days of vancomycin therapy is usually sought before decisions are made for therapeutic changes. Last, our studies evaluated the pharmacodynamic effects of telavancin against only 4 isolates that were all susceptible to vancomycin. Therefore, assessment of the effects of telavancin against isolates that are tolerant, heteroresistant, or nonsusceptible to vancomycin after vancomycin exposure is still needed.

To our knowledge, this is the first study to assess the potential impact of prior vancomycin exposure on telavancin therapy against MRSA and MSSA. Given the lack of difference in the microbiological outcome between telavancin and vancomycin, as well as the lack of development of resistance against telavancin after vancomycin exposure, the findings of this study suggest that telavancin activity is unaffected by prior vancomycin exposure. Further studies in clinical practice and with additional isolates are warranted to support these observations.

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