

## Telavancin and vancomycin pharmacodynamics with *Staphylococcus aureus* in an *in vitro* dynamic model

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**Objectives:** The aim of this study was to compare the pharmacodynamics of telavancin (TLV) and vancomycin (VAN) with *Staphylococcus aureus*. Their concentrations were simulated between the MIC and the mutant prevention concentration (MPC), and above the MPC.

**Methods:** Two strains of *S. aureus*, glycopeptide-intermediate *S. aureus* (GISA) Mu-50 and ATCC 43300, were exposed for 5 days to once-daily TLV (half-life 8 h) and twice-daily VAN (half-life 6 h). The simulated ratios of 24 h area under the curve (AUC<sub>24</sub>) to MIC varied from 30–50 to 3400 h. The cumulative antimicrobial effect was expressed by ABBC (area between the level corresponding to the starting inoculum and the time–kill curve calculated from time 0 to 144 h).

**Results:** With each antibiotic, the ABBC versus log AUC<sub>24</sub>/MIC relationships were bacterial strain-independent. A sigmoid model fits combined data on both organisms exposed to TLV ( $r^2=0.78$ ) or VAN ( $r^2=0.85$ ). Comparable effects of the proposed therapeutic dose of TLV (10 mg/kg) and a clinical dose of VAN (2×1 g) were predicted for MRSA ATCC 43300 (AUC<sub>24</sub>/MIC 3400 and 500 h, respectively) and a 1.6-fold greater effect of TLV for GISA Mu-50 compared with VAN (AUC<sub>24</sub>/MIC 1700 and 130 h, respectively). Mutants of *S. aureus* ATCC 43300 resistant to 2× and 4× MIC of VAN but not TLV were enriched in these simulations. No selection of TLV- and VAN-resistant mutants of GISA Mu-50 was observed.

**Conclusions:** These *in vitro* data suggest that the effects of clinically attainable AUC/MIC ratios of TLV are similar to those of VAN on *S. aureus* 43300 and 2-fold greater on GISA Mu-50.

Keywords: telavancin, vancomycin, *S. aureus*, pharmacodynamics, *in vitro* model

### Introduction

Telavancin, a semi-synthetic derivative of vancomycin, is a novel lipoglycopeptide with rapid bactericidal activity and multiple mechanisms of action against Gram-positive bacteria, including methicillin-resistant, glycopeptide-intermediate and vancomycin-resistant strains of *Staphylococcus aureus*.<sup>1–3</sup> Unlike other glycopeptides, telavancin not only inhibits biosynthesis of bacterial cell wall peptidoglycan, but also induces enhanced permeability of the bacterial cell membrane and dissipation of the membrane potential. These events correlate temporally with a potentially higher barrier for resistance development.<sup>4</sup>

Only a few time–kill studies report the activities of telavancin against *S. aureus*. One such study exposed

glycopeptide-intermediate *S. aureus* (GISA) and vancomycin-resistant *S. aureus* (VRSA) to constant antibiotic concentrations.<sup>5</sup> In this study, telavancin, vancomycin, daptomycin and linezolid had similar activity on GISA strains, and telavancin had greater effects compared with vancomycin on VRSA strains. Another *in vitro* study exposed methicillin-resistant *S. aureus* (MRSA) to changing concentrations of telavancin that simulated its single-dose pharmacokinetics.<sup>6</sup> Telavancin displayed potent antibacterial activity against the studied organisms, but the non-comparative design of this study did not allow direct comparison of telavancin with other antistaphylococcal agents.

The goals of the present study were to compare the pharmacodynamics of telavancin and vancomycin on MRSA

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(vancomycin-susceptible) and GISA, and to establish the concentration–response relationships for these two agents in these two strains.

## Materials and methods

### Antimicrobial agents, bacterial strains and susceptibility testing

Telavancin, kindly provided by Theravance, Inc. (San Francisco, CA, USA), and vancomycin (MP Biomedicals, Inc., Solon, CA, USA) were used in the study.

Two MRSA strains, vancomycin-susceptible *S. aureus* ATCC 43300 and GISA Mu-50, were selected for the study.

Susceptibility testing was performed in triplicate by broth micro-dilution techniques at 24 h post-exposure with the organism grown in  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -supplemented Mueller–Hinton broth (MHB) at an inoculum size of  $10^6$  cfu/mL. With MRSA and GISA, the MICs of telavancin were estimated at 0.25 and 0.5 mg/L, respectively, and the MICs of vancomycin were 0.78 and 3.12 mg/L, respectively. To reveal possible changes in susceptibility of antibiotic-exposed staphylococci, MICs were determined prior to, during and after a 5 day period of treatment.

Mutant prevention concentrations (MPCs) were determined as described elsewhere.<sup>7</sup> Briefly, the tested microorganisms were cultured in MHB and incubated for 24 h. The suspension was centrifuged (4000 g for 10 min) and re-suspended in MHB to yield a concentration of  $10^{11}$  cfu/mL. A series of agar plates containing known antibiotic concentrations was then inoculated with  $\sim 10^{11}$  cfu of *S. aureus*. The inoculated plates were incubated for 48 h at 37°C and visually screened for growth. To estimate the MPC, logarithms of bacterial numbers were plotted against antibiotic concentrations. MPC was taken as the point where the plot intersects the theoretical limit of detection ( $\log \text{cfu/mL} = 1$ ). The MPCs of telavancin and vancomycin for MRSA were estimated at 4.7 and 15 mg/L, and those for GISA Mu-50 were 12 and 21 mg/L, respectively.

### Simulated pharmacokinetic profiles

Mono-exponential concentration decays of telavancin (as a single dose) and vancomycin (as two 12 hourly doses) were simulated for 5 consecutive days with half-lives of 8 and 6 h, respectively, in accordance with the values reported in humans.<sup>3,8</sup> To provide peak concentrations of each antibiotic between the MIC and the MPC [i.e. within the mutant selection window (MSW)] and above the MPC, the steady-state ratios of 24 h AUC ( $\text{AUC}_{24}$ ) to MIC were varied from 30–50 to 200 h and from 400 to 3400 h, respectively. Peak concentrations ( $C_{\text{max}}$ ) of the antibiotics were located between the MICs and the MPCs (i.e. within the MSWs), and 2- to 16-fold higher than the MPCs. All experiments were performed at least in duplicate.

### In vitro dynamic model

A previously described dynamic model<sup>9</sup> was used in the study. Briefly, the model consisted of two connected flasks, one containing fresh MHB and the other a magnetic stirrer, the central unit, and the same broth containing either a bacterial culture alone (control growth experiments) or a bacterial culture plus antibiotic (killing/regrowth experiments). With telavancin, peristaltic pumps circulated fresh nutrient medium to the flasks and from the central 75 mL unit at a flow rate of 6.5 mL/h. With vancomycin, culture-containing medium in a 60 mL unit was diluted at a flow rate of 7 mL/h.

The system was filled with sterile MHB and placed in an incubator at 37°C. The central unit was inoculated with an 18 h culture of *S. aureus* to approach  $\sim 10^8$  cfu/mL. After 30 min of incubation, telavancin or vancomycin was injected into the central unit.

### Quantification of the antimicrobial effect and susceptibility changes

In each experiment, multiple sampling of bacteria-containing media from the central compartment was performed throughout the observation period. One hundred microlitre samples were plated onto Mueller–Hinton agar plates. In order to account for antibiotic carry-over, all samples were diluted sufficiently prior to plating, therefore reducing the antibiotic concentration below the MIC of the drug. The lower limit of accurate detection was  $20 \times 10^2$  cfu/mL.

To detect changes in susceptibility, each 24 h sample was plated onto agar plates containing  $2 \times$  and  $4 \times$  MIC of telavancin or vancomycin (detection limit 10 cfu/mL). In addition, the MICs of each antibiotic were determined prior to and after treatment.

The duration of the experiments was defined as the time until antibiotic-exposed bacteria (after the last dose) reached the inoculum size.

To determine the cumulative antimicrobial effect, the area between the upper limit of bacterial numbers and the time–kill curve (ABBC)<sup>10</sup> was calculated from time 0 to 144 h.

### Relationships of the antimicrobial effect to $\text{AUC}_{24}/\text{MIC}$

The antimicrobial effect was related to  $\log (\text{AUC}_{24}/\text{MIC})$  and fitted by the Hill equation:

$$Y = Y_{\text{max}} x^n / [(x_{50})^n + x^n] \quad (1)$$

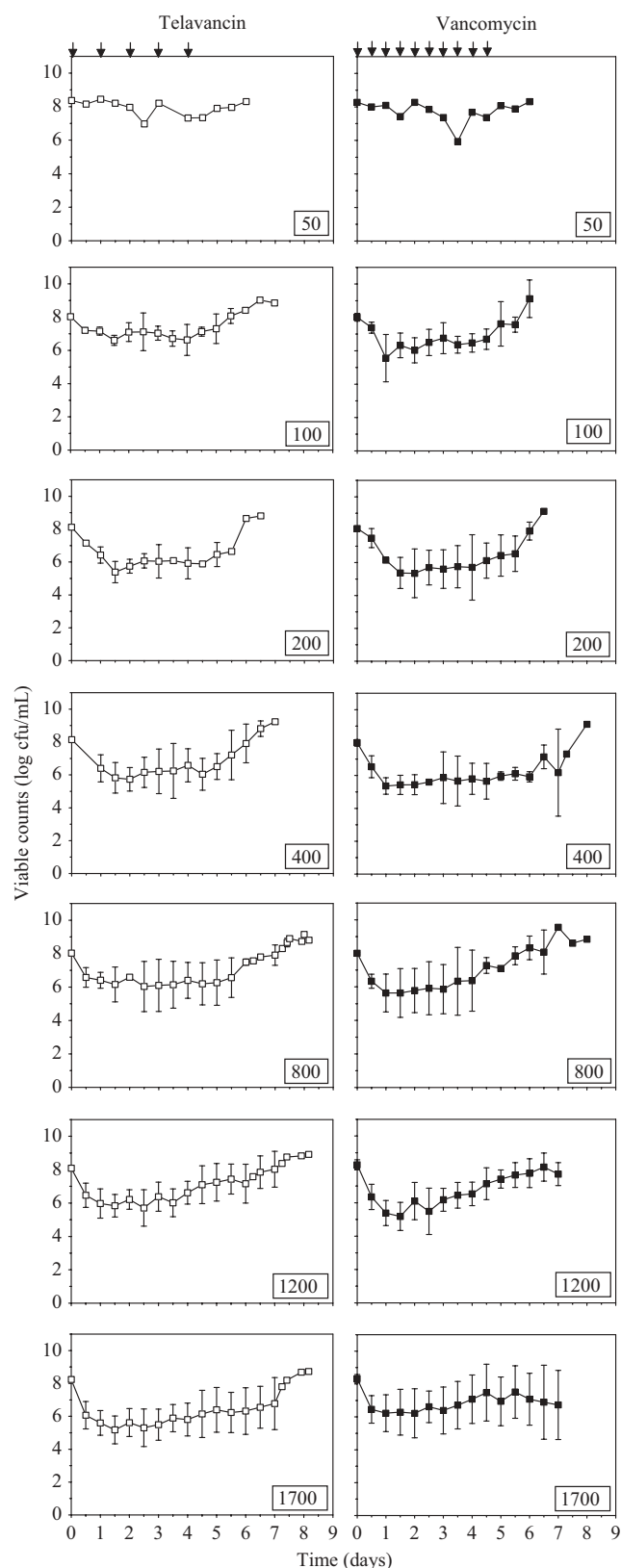
where  $Y$  is ABBC,  $x$  is  $\text{AUC}_{24}/\text{MIC}$ ,  $Y_{\text{max}}$  is the maximal value of ABBC,  $x_{50}$  is  $\text{AUC}_{24}/\text{MIC}$  that provides the antimicrobial effect equal to  $Y_{\text{max}}/2$  and  $n$  is a parameter.

## Results

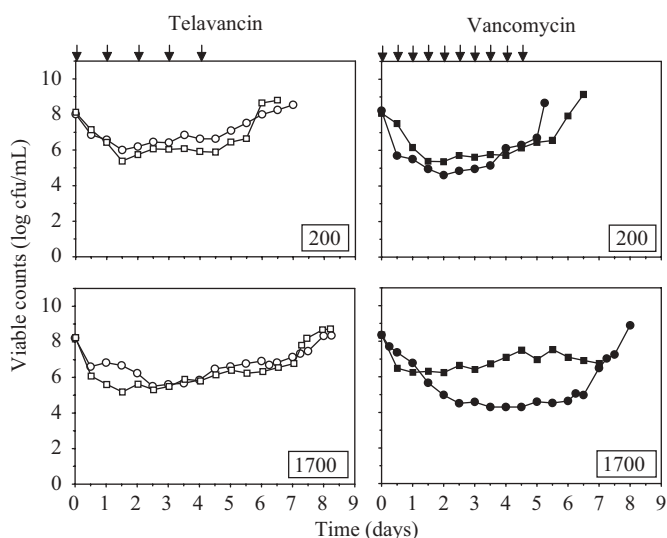
Time–kill curves of GISA Mu-50 exposed to telavancin and vancomycin are shown in Figure 1. As seen in the figure, antibiotic-induced reduction of the starting inoculum occurred with each treatment, except for the lowest simulated  $\text{AUC}_{24}/\text{MIC}$  ratio (50 h). At  $\text{AUC}_{24}/\text{MIC}$ s above 50 h but below 800 h, the extent of bacterial killing was concentration-dependent: the greater the  $\text{AUC}_{24}/\text{MIC}$  ratio, the more pronounced the reduction of the starting inoculum. Further increase in the simulated  $\text{AUC}_{24}/\text{MIC}$  ratio up to 3400 h did not lead to further decrease in the minimal numbers of survivors. Similar killing kinetics were observed with telavancin- and vancomycin-exposed *S. aureus* ATCC 43300 (data not shown).

There were no differences in telavancin effects on *S. aureus* ATCC 43300 and GISA Mu-50 over the entire range of the simulated  $\text{AUC}_{24}/\text{MIC}$  ratio. For example, at both  $\text{AUC}_{24}/\text{MIC}$ s of 200 and 1700 h (Figure 2, left-hand panels), the respective time–kill curves were practically superimposed. Unlike telavancin, vancomycin-induced killing of GISA Mu-50 was less pronounced than that of *S. aureus* ATCC 43300 at the higher, but not the lower  $\text{AUC}_{24}/\text{MIC}$  (Figure 2, right-hand panels). Plotting ABBCs versus  $\text{AUC}_{24}/\text{MIC}$ s for ATCC 43300 and

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**Figure 1.** Time–kill curves of GISA Mu-50 exposed to telavancin and vancomycin. Antibiotic dosing is indicated by the arrows, and simulated  $AUC_{24}/MIC$  ratio is indicated by the boxed number in each plot. Viable counts are plotted as the means plus standard deviations of results from at least three separate experiments.



**Figure 2.** Comparative killing/regrowth kinetics of *S. aureus* ATCC 43300 (circles) and GISA Mu-50 (squares) exposed to telavancin (open symbols) and vancomycin (filled symbols) at the same  $AUC/MIC$  ratios.

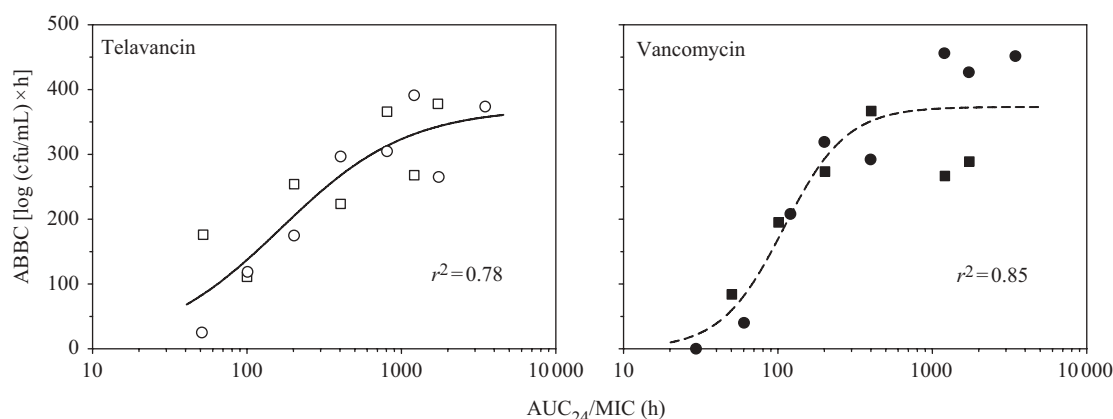
Mu-50 exposed to telavancin (Figure 3, left-hand panel) or vancomycin (Figure 3, right-hand panel) revealed no systematic differences between the effects of each antibiotic on the organisms. As seen in the figure, the Hill equation fits these combined data obtained with telavancin ( $r^2 = 0.78$ ) or vancomycin ( $r^2 = 0.85$ ). According to the model, half of the maximal effect can be provided by the  $AUC_{24}/MIC$  ratio of vancomycin, which is lower than that of telavancin ( $x_0$  109 versus 166 h). However, the maximal attainable effects of the antibiotics are similar:  $Y_{max} = 373$  and  $372$  ( $\log [cfu/mL] \times h$ ), respectively.

Regardless of whether telavancin or vancomycin concentrations were within or beyond the MSW, no bacterial growth of GISA Mu-50 occurred on antibiotic-containing media. Mutants of *S. aureus* ATCC 43300 resistant to  $2\times$  and  $4\times$  MIC of vancomycin but not telavancin were enriched at  $AUC_{24}/MIC$  of 60 and 120 h. None of the simulated regimens led to a loss in susceptibility of telavancin- or vancomycin-exposed staphylococci.

## Discussion

This is the first study using multiple-dose *in vitro* simulations of telavancin to show the  $AUC_{24}/MIC$ -dependent antistaphylococcal effects of the antibiotic, without selection of resistant mutants or loss in susceptibility.

For both organisms, MRSA ATCC 43300 and GISA Mu-50, an increase in the simulated  $AUC_{24}/MIC$  ratio for telavancin from 30–50 to 400–800 h was accompanied by gradually increasing the cumulative antimicrobial effects (ABBC). Further increases in the ABBC were not observed at higher  $AUC_{24}/MIC$ s (up to 3400 h). Similar saturation of the antistaphylococcal effect has been reported in an *in vitro* study that simulated single-dose telavancin pharmacokinetics.<sup>6</sup> In fact, concentration-dependent 24 h reductions of the starting inoculum were observed at the relatively low initial antibiotic concentrations (from 1 to 5 mg/L), but not at higher concentrations (from 5 to 40 mg/L). The saturable pattern of telavancin's *in vitro* antistaphylococcal effects is consistent with the findings obtained in a



**Figure 3.** AUC<sub>24</sub>/MIC relationships of the antistaphylococcal effect fitted by Equation (1). Telavancin against *S. aureus* ATCC 43300 (open circles) and GISA Mu-50 (open squares):  $x_{50} = 166 \pm 72$ ,  $Y_{\max} = 372 \pm 56$ ,  $n = 1.1 \pm 0.4$ . Vancomycin against *S. aureus* ATCC 43300 (filled circles) and *S. aureus* Mu-50 (filled squares):  $x_{50} = 109 \pm 20$ ,  $Y_{\max} = 373 \pm 27$ ,  $n = 2.1 \pm 0.8$ .

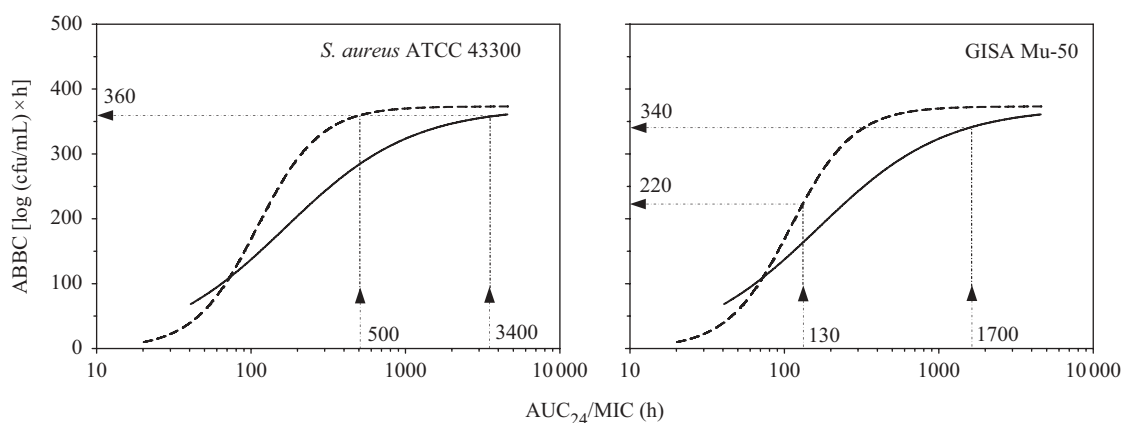
rabbit endocarditis model:<sup>11</sup> similar bacteriological efficacies of the relatively high AUC/MICs (160 h with *S. aureus* HIP 5836 and 640 h with GISA Mu-50) were observed in these simulations of human-like pharmacokinetics.

In the present study, the antistaphylococcal effects of telavancin and vancomycin on MRSA ATCC 43300 and GISA Mu-50 observed at a given AUC<sub>24</sub>/MIC ratio were comparable, although at the highest simulated AUC<sub>24</sub>/MIC (1700 h), the extent of killing of telavancin-exposed GISA Mu-50 was greater than with vancomycin. Due to the similar pharmacodynamics of both telavancin and vancomycin with MRSA ATCC 43300 and GISA Mu-50, the Hill equation fits the combined data on both organisms exposed to either telavancin ( $r^2 = 0.78$ ) or vancomycin ( $r^2 = 0.85$ ). Based on this model, comparable effects of the proposed therapeutic dose of telavancin (10 mg/kg) and a clinical dose of vancomycin (2 × 1 g) can be predicted for MRSA ATCC 43300 (AUC<sub>24</sub>/MIC 3400 and 500 h, respectively) (Figure 4, left-hand panel). However, a 1.6-fold greater effect of telavancin is predicted for GISA Mu-50 compared with vancomycin (AUC<sub>24</sub>/MIC 1700 and 130 h, respectively) (Figure 4, right-hand panel).

When making these predictions, we did not refer to unbound antibiotic concentrations and ‘free’ AUCs that can easily be calculated from reported data on the protein binding of telavancin and vancomycin (~90%<sup>3</sup> and 42%<sup>12</sup> to 55%<sup>13</sup>, respectively).

As shown earlier,<sup>14</sup> this simplified approach to the protein-binding effects on antibiotic activity can be misleading: binding effects may be overestimated and true antimicrobial effects underestimated. This may also apply to the interpretation of telavancin’s pharmacodynamics, which better relate to total rather than free concentrations. For example, both the greater bacteriological efficacy of telavancin, and the better survival of mice with staphylococcal bacteraemia, compared with vancomycin<sup>15</sup> can be explained by the higher total AUC<sub>24</sub>/MIC ratio (2200 versus 225 h) rather than the similar free AUC<sub>24</sub>/MICs (126 versus 130 h). The analysis of total antibiotic concentration appeared more relevant than that based on free telavancin concentrations in another study in infected neutropenic mice.<sup>16</sup> Again, an attempt to relate the antistaphylococcal effects of free concentrations of telavancin was disappointing: pronounced reductions in bacterial titres were observed at ‘free’ concentrations below the MIC throughout the dosing interval (time above MIC of 0). These findings imply that the true effects of protein binding on the efficacy of telavancin are much less than expected. Indeed, minimal, if any, differences in killing of telavancin-exposed staphylococci, with and without albumin, were reported in the single-dose *in vitro* dynamic model study cited above.<sup>6</sup>

Overall, this study suggests that the antistaphylococcal effects of telavancin and vancomycin are concentration-dependent but



**Figure 4.** Prediction of the antistaphylococcal effects of telavancin (continuous line) and vancomycin (broken line) at clinically attainable AUC<sub>24</sub>/MIC ratios.

saturable at higher AUC<sub>24</sub>/MICs. The effects of clinically attainable AUC<sub>24</sub>/MIC ratios of telavancin are similar to vancomycin in MRSA ATCC 43300, but 1.6-fold greater for telavancin versus vancomycin in GISA Mu-50.

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## Transparency declarations

None to declare.

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