Testing the mutant selection window hypothesis with Staphylococcus aureus exposed to daptomycin and vancomycin in an in vitro dynamic model

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Objectives: To extend the mutant selection window (MSW) hypothesis to include antibiotics in addition to fluoroquinolones, the pharmacodynamics of daptomycin (DAP) and vancomycin (VAN) and their ability to prevent the selection of resistant Staphylococcus aureus were studied in an in vitro model that simulates antibiotic concentrations below the MIC, between the MIC and the mutant prevention concentration (MPC), and above the MPC.

Methods: Two clinical isolates of S. aureus, S. aureus 866 (MICDAP 0.35, MICVAN 0.7, MPCDAP 1.1, MPCVAN 2.4 mg/L) and S. aureus 10 (MICDAP 1.1, MICVAN 1.3, MPCDAP 5.5, MPCVAN 11 mg/L), were exposed for five consecutive days to once-daily daptomycin (half-life 9 h) and twice-daily vancomycin (half-life 6 h) at the ratio of 24 h area under the concentration–time curve (AUC24) to MIC that varied over a 16- to 30-fold range. The cumulative antimicrobial effect was expressed by its intensity (IE). Changes in susceptibility and numbers of surviving organisms on agar plates containing 2·MIC and 4·MIC of daptomycin or vancomycin were monitored daily.

Results: The IE-log AUC24/MIC plots were bacterial strain- and antibiotic-independent. This allowed combination of data obtained with both antibiotics and both organisms. Based on the sigmoid relationship between IE and the AUC24/MIC (r² = 0.9), the antistaphylococcal effect of the therapeutic doses of daptomycin (4 and 6 mg/kg) against a hypothetical S. aureus with MIC equal to the MIC90 (AUC24/MIC90 380 and 570 h, respectively) was predicted to be similar to the effect of two 1 g doses of vancomycin given at a 12 h interval (AUC24/MIC90 200 h). AUC24/MIC relationships of the final-to-initial MIC ratio and logarithm of the ratio of maximal-to-initial numbers of organisms resistant to 2·MIC or vancomycin were bell-shaped and bacterial strain- and antibiotic-independent. Based on these relationships, an AUC24/MIC ratio that protects against the selection of resistant mutants was predicted at 1/C21. This protective value is less than the AUC24/MIC90s provided by the 4 mg/kg dose and considerably less than the 6 mg/kg dose of daptomycin, but it is close to the AUC24/MIC90 provided by two 1 g doses of vancomycin.

Conclusions: These findings support the MSW hypothesis and suggest comparable antistaphylococcal effects of clinically achievable AUC24/MIC90s of daptomycin and vancomycin but slightly better prevention against the selection of resistant S. aureus by daptomycin.

Keywords: S. aureus, pharmacodynamics, resistance, in vitro model

Introduction
The hypothesis of the mutant selection window (MSW), that is the concentration range from the MIC to the mutant prevention concentration (MPC), within which it is proposed that resistant mutants are enriched or selected1 has passed the test successfully with Staphylococcus aureus2–6 and Streptococcus pneumoniae7 exposed to fluoroquinolones in dilution2,4–6 and hollow-fibre
in vitro models. Despite the use of different dynamic models, the most pronounced loss in susceptibility of antibiotic-exposed organisms and the enrichment of resistant mutants were reported over a comparable range of simulated ratios of 24 h area under the concentration–time curve (AUC24) to MIC (from 25 to 100–150 h), when antibiotic concentrations fell into the MSW for most of the dosing interval.

To extend the MSW hypothesis beyond fluoroquinolones, two strains of S. aureus were exposed to daptomycin and vancomycin pharmacokinetics at concentrations below the MICs, between the MICs and MPCs, and above the MPCs in a dynamic model that simulates 5 day treatments with the two antibiotics.

Materials and methods

Antimicrobial agents, bacterial strain and susceptibility testing

Daptomycin and vancomycin were kindly provided by Cubist Pharmaceuticals, Inc. (Lexington, MA, USA) and MP Biomedicals, Inc. (Solon, CA, USA), respectively.

Two clinical isolates of S. aureus, that is S. aureus 866 (methicillin-resistant S. aureus, MRSA) and S. aureus 10 (methicillin-susceptible S. aureus, MSSA) were selected for the study. Susceptibility testing was performed in triplicate by broth microdilution techniques at 24 h post-exposure with the organism grown in Mueller–Hinton broth (MHB) at an inoculum size of 10^8 cfu/mL. Because daptomycin antimicrobial activity is influenced by the presence of Ca^{2+}, MHB supplemented with 50 mg of Ca^{2+}/L was used for all susceptibility studies. With S. aureus 866 and S. aureus 10, the MICs of daptomycin were estimated at 0.35 and 1.1 mg/L, respectively, and the MICs of vancomycin were 0.7 and 1.3 mg/L, respectively. To reveal possible changes in susceptibility of antibiotic-exposed staphylococci, the MICs were determined prior, during and after a 5 day treatment.

The MPCs were determined as described elsewhere. Briefly, the tested microorganisms were cultured in MHB and incubated for 24 h. Then, the suspension was centrifuged (4000 g for 10 min) and resuspended in MHB to yield a concentration of 10^11 cfu/mL. A series of agar plates containing known antibiotic concentrations was then inoculated with ~10^11 cfu of S. aureus. The inoculated plates were incubated for 48 h at 37°C and screened visually for growth. To estimate the MPC, logarithms of bacterial numbers were plotted against antibiotic concentrations (Figure 1). MPC was taken as the point where the plot intersected the theoretical limit of detection (log cfu/mL = 1).

The MPCs of daptomycin and vancomycin were estimated at 1.1 and 2.4 mg/L for S. aureus 866, and 5.5 and 11 mg/L for S. aureus 10, respectively.

Simulated pharmacokinetic profiles

Mono-exponential concentration decays of daptomycin (as a single dose) and vancomycin (as two 12 hourly doses) were simulated for five consecutive days with half-lives of 9 and 6 h, respectively, in accordance with values reported in humans. With S. aureus 866 exposed to both antibiotics and with daptomycin-exposed S. aureus 10, the simulated AUC24/MIC ratios were 16, 32, 64, 128 and 256, and with S. aureus 10 exposed to vancomycin the simulated AUC24/MIC ratios were 13, 26, 54, 108, 216 and 432 h. Peak concentrations (Cmax values) of the antibiotics were equal to the MIC, located between the MIC and the MPC, that is within the MSW, and above the MPC. With S. aureus 866 exposed to both antibiotics and daptomycin-exposed S. aureus 10, the steady-state Cmax/MIC ratios varied from 1.2 to 20.0 and with S. aureus 10 exposed to vancomycin the ratios varied from 1.5 to 50. Most experiments were performed at least in duplicate.

In vitro dynamic model

A previously described dynamic model was used in this study. Briefly, the model consisted of two connected flasks, one containing fresh MHB supplemented with 50 mg of Ca^{2+}/L (daptomycin experiments) and the other with a magnetic stirrer, the central unit, with the same broth containing either a bacterial culture alone or a bacterial culture plus antibiotic (killing/regrowth experiments). Peristaltic pumps circulated fresh nutrient medium to the flasks and from the central 60 mL unit at a flow rate of 4.6 mL/h with daptomycin and 6.9 mL/h with vancomycin. The reliability of antibiotic pharmacokinetic simulations and the high reproducibility of the time–kill curves provided by the model have been reported elsewhere.

The system was filled with sterile MHB and placed in an incubator at 37°C. The central unit containing 54 mL of fresh MHB was inoculated with 6 mL of an 18 h culture of S. aureus (10^7 cfu/mL). After a 30 min incubation daptomycin or vancomycin was injected into the central unit.

Quantification of the antimicrobial effect and susceptibility changes

In each experiment, multiple sampling of bacteria-containing medium from the central compartment was performed throughout the observation period. Samples (100 µL) were serially diluted as appropriate, and 100 µL was plated onto Mueller–Hinton agar plates. The duration of the experiments was defined in each case as the time after the last dose until antibiotic-exposed bacteria reached the maximum numbers observed in the absence of antibiotic (≥10⁹ cfu/mL). The lower limit of accurate detection was 2×10^⁵ cfu/mL.

Based on time–kill data, the intensity of the antimicrobial effect (I) was determined from time zero to the time at which the number of antibiotic-exposed bacteria reached 10⁹ cfu/mL. The computation of I is depicted graphically in Figure 2.

To reveal possible changes in susceptibility during treatment, MICs for bacterial cultures sampled from the model were determined.

Figure 1. Determination of MPC: estimated values are indicated by italicized numbers. Daptomycin against S. aureus 866 (squares) and S. aureus 10 (triangles); vancomycin against S. aureus 866 (diamonds) and S. aureus 10 (inverted triangles).
where $x = \frac{MIC_{\text{final}}}{MIC_{\text{initial}}}$, and $E = f(x)$.

**Equation 1**

The $I_E$-log AUC$_{24}$/MIC curve was fitted by the logistic function:

$$E = E_{\text{min}} + \left( E_{\text{max}} - E_{\text{min}} \right) \left[ 1 + \left( x/x_0 \right)^2 \right]^{-1}$$

where $x$ is the AUC$_{24}$/MIC ratio, $E$ is $I_E$, $E_{\text{max}}$ and $E_{\text{min}}$ are the maximal and minimal values of the antimicrobial effect, $x_0$ is $x$ corresponding to $(E_{\text{min}} + E_{\text{max}})/2$ and is a parameter reflecting sigmoidicity.

**Relationships of the emergence of resistance to AUC$_{24}$/MIC**

To relate both the enrichment of resistant mutants [expressed as the logarithm of the ratio of maximal number ($N_{\text{max}}$) to the initial number ($N_{\text{initial}}$) of organisms resistant to $2x$ and $4x$ MIC of daptomycin or vancomycin] and the increase in MIC [expressed as the final-to-initial MIC ratio ($MIC_{\text{final}}/MIC_{\text{initial}}$)] to the simulated AUC$_{24}$/MICs, a Gaussian-type function was used:

$$Y = Y_0 + a \exp\left[ -0.5 \left( x - x_c \right)^2/b^2 \right]$$

where $Y$ is the $MIC_{\text{final}}/MIC_{\text{initial}}$ ratio or log $N_{\text{max}}/N_{\text{initial}}$, $Y_0$ is the minimal value of $Y$, $x$ is the log AUC$_{24}$/MIC, $x_c$ is the log AUC$_{24}$/MIC that corresponds to the maximal value of log $N_{\text{max}}/N_{\text{initial}}$ or $MIC_{\text{final}}/MIC_{\text{initial}}$, and $a$ and $b$ are parameters. Equation 2 was also used to fit $N_{\text{max}}/N_{\text{initial}}$ or $MIC_{\text{final}}/MIC_{\text{initial}}$ versus AUC/MPC data.

To relate the emergence of resistance with the time during which antibiotic concentrations are within the MSW ($T_{\text{MSW}}$), the log $N_{\text{max}}/N_{\text{initial}}$ or $MIC_{\text{final}}/MIC_{\text{initial}}$ ratio was fitted to the $T_{\text{MSW}}$ using Equation 1, where $E$ is either log $N_{\text{max}}/N_{\text{initial}}$ or the $MIC_{\text{final}}/MIC_{\text{initial}}$, $x$ is $T_{\text{MSW}}$ and $x_0$ is the $T_{\text{MSW}}$ that corresponds to $(E_{\text{min}} + E_{\text{max}})/2$.

**Results**

**Pharmacodynamics**

The time courses of killing and regrowth of *S. aureus* 866 exposed to daptomycin and vancomycin are shown in Figure 3. The lowest daily for 5 days. Moreover, each sample was plated onto agar plates containing $2x$ and $4x$ MIC of daptomycin or vancomycin.

**Figure 2.** Determination of $I_E$ (shaded area): killing of *S. aureus* 866 exposed to daptomycin. Antibiotic dosing is indicated by the arrows.

**Figure 3.** Time-kill curves of *S. aureus* 866 exposed to daptomycin (squares, top panel) and vancomycin (diamonds, bottom panel). Antibiotic dosing is indicated by the arrows, and simulated AUC$_{24}$/MIC ratios are indicated next to each plot.

Simulated AUC$_{24}$/MIC ratio (16 h) with $C_{\text{max}}$/MICs close to the MICs did not reduce the starting inoculum. The higher AUC$_{24}$/MICs, with daptomycin and vancomycin $C_{\text{max}}$/MICs exceeding the MICs over a considerable part of the dosing interval (AUC$_{24}$/MIC 32 h) or the entire dosing interval (AUC$_{24}$/MIC 64–256 h), resulted in pronounced reductions in bacterial counts, although regrowth occurred by the end of each treatment. In general, an increase in the simulated AUC$_{24}$/MIC ratio led to the lower minimal numbers of surviving organisms and to later regrowth. Similar AUC$_{24}$/MIC-dependent killing was observed with daptomycin- and vancomycin-exposed *S. aureus* 10 (data not shown).

Plotting $I_E$ versus log AUC$_{24}$/MIC (Figure 4) gives a sigmoid curve that is not specific for antibiotic or for bacterial strain: Equation 1 fits combined data obtained with each antibiotic–pathogen pair. Given the strain-independent pattern of the $I_E$-log AUC$_{24}$/MIC relationship, it can predict daptomycin and vancomycin effects on a hypothetical strain of *S. aureus*, for example, a strain with MICs equal to the MIC$_{90}$ (1 and 2 mg/L, respectively). As seen in the figure, at the clinically achievable AUC$_{24}$/MIC$_{90}$ ratios (380 and 570 h for 4 and 6 mg/kg daptomycin, respectively, and 200 h for 2x 1 g vancomycin), the predicted effects of both antibiotics are quite similar.
Emergence of resistance

Figures 5 and 6 present the time courses of numbers of surviving bacteria on daptomycin- or vancomycin-containing agar plates (2x and 4x MIC) and the concomitant changes in susceptibility at antibiotic concentrations within or out of the MSWs over most of the dosing interval (three of five or six dosing regimens simulated for each antibiotic–pathogen pair are shown). As seen in Figure 5, at both the lowest (AUC24/MIC 16 h) and the highest (AUC24/MIC 256 h) concentrations, no mutant of S. aureus 866 resistant to 2x and 4x MIC of daptomycin or vancomycin was selected, and no loss in susceptibility occurred. At AUC24/MIC of 32 h, when antibiotic concentrations fell into the MSWs, the population was enriched with resistant mutants, and the susceptibility of bacteria sampled from the model decreased gradually. Similar data were obtained with S. aureus 10 (Figure 6): no selection of resistant mutants at AUC24/MIC of 13–16 h and 216–256 h in contrast to the pronounced selection at AUC24/MIC of 54–64 h.

Based on data obtained over the entire simulated AUC24/MIC ranges (five or six AUC24/MICs with each antibiotic–pathogen pair), ratios of the maximal to the initial bacterial numbers on plates containing 2x or 4x MIC and ratio of the final-to-initial MICs were plotted against the simulated AUC24/MICs. As seen in Figure 7, both log Nmax/Ninitial and MICfinal/MICinitial ratios were AUC24/MIC-dependent in a bell-shaped fashion. The respective Gaussian relationships (Equation 2) were not specific for antibiotic or bacterial strain (r²s 0.64–0.68). Moreover, regardless of the method used to demonstrate resistance (population analysis or susceptibility testing), maximal enrichment of resistant mutants and maximal loss in susceptibility occurred at similar AUC24/MIC ratios (50 and 48 h, respectively). Furthermore, an AUC24/MIC ratio that protects against the selection of resistant mutants appears to be the same for both antibiotics: C21/C2MIC 200 h. This value corresponds to the clinically achievable AUC 24/MIC90.
Daptomycin pharmacodynamics with staphylococci

Figure 6. In vitro simulated pharmacokinetics (a), and time courses of S. aureus 10 that survived on antibiotic-containing plates with 2× MIC (b) and 4× MIC (c) and those of susceptibility to daptomycin and vancomycin (d)—selected data. Antibiotic dosing is indicated by the arrows, and simulated AUC24/MIC ratios are indicated by boxed numbers.

provided by 2× 1 g vancomycin (200 h) but it is 2- to 3-fold less than what is provided by 4 and 6 mg/kg daptomycin (380 and 570 h, respectively).

To relate the observed selection of resistant staphylococci or the lack of such selection to the time period when antibiotic concentrations are within the MSW, log \(N_{\text{max}}/N_{\text{initial}}\) (on plates containing 2× MIC of daptomycin or vancomycin) and MIC\(_{\text{final}}/\text{MIC}_{\text{initial}}\) were plotted against \(T_{\text{MSW}}\) (Figure 8). \(T_{\text{MSW}}\) plots were sigmoid and fitted by Equation 1 with smaller \(r^2\)s (0.5–0.6) than those established for the respective AUC24/MIC plots. An even looser correlation was established between \(T_{\text{MSW}}\) and log \(N_{\text{max}}/N_{\text{initial}}\) on plates containing 4× MIC of antibiotic (\(r^2 = 0.3\); data not shown).

**Discussion**

The MSW hypothesis was shown to be relevant to the studied lipopeptide and glycopeptide antibiotics: both selection of organisms resistant to 2× and 4× MIC of daptomycin or vancomycin and decreased susceptibility of staphylococci occurred at antibiotic concentrations that fell into the MSWs. Antibiotic- and bacterial strain-independent bell-shaped relationships between MIC\(_{\text{final}}/\text{MIC}_{\text{initial}}\) and AUC24/MIC were similar to those reported in in vitro studies with fluoroquinolones\(^5\)–\(^7\) although AUC24/MIC plots of resistance of S. aureus to daptomycin and vancomycin were more scattered. However, maximal enrichment of resistant mutants and significant loss in susceptibility of daptomycin- or vancomycin-exposed S. aureus were observed at AUC24/MIC ratios (30–60 h) that are comparable with those reported with the fluoroquinolones and S. aureus: from 25 to 100 h\(^6\) and from 30 to 150 h\(^3\) for ciprofloxacin, from 25 to 100 h for gatifloxacin,\(^4\) levofloxacin\(^5\) and moxifloxacin\(^6\) and from 60 to 120 h for ABT-492,\(^5\) but not with findings reported in a recent study with levofloxacin-exposed staphylococci,\(^15\) where the enrichment of resistant mutants was observed only at an AUC24/MIC of 30 h but not at 60 h.

Recently, the ratio of AUC to MPC of ciprofloxacin was suggested to be a better predictor of the enrichment of resistant Escherichia coli than AUC/MIC,\(^16\) although this statement was not entirely supported by the presented data. In the present study, neither MIC\(_{\text{final}}/\text{MIC}_{\text{initial}}\) (Figure 9) nor \(N_{\text{max}}/N_{\text{initial}}\) (data not shown) correlated with the AUC24/MPC ratio. Further studies examining AUC24/MPC relationships of resistance with additional antibiotics are needed to compare different predictors of resistant mutant enrichment.

Unlike our earlier findings with fluoroquinolones,\(^6\) less accurate relationships were established in this study when log \(N_{\text{max}}/N_{\text{initial}}\) or MIC\(_{\text{final}}/\text{MIC}_{\text{initial}}\) were plotted against \(T_{\text{MSW}}\). At first glance, this is consistent with the unsuccessful attempts to relate resistance of ciprofloxacin-exposed S. aureus to \(T_{\text{MSW}}\) as reported by Campion et al.\(^3\) However, this similarity is more apparent than real. Looking closely at the MIC\(_{\text{final}}/\text{MIC}_{\text{initial}}\) versus \(T_{\text{MSW}}\) data reported in the ciprofloxacin study, a reasonable \(T_{\text{MSW}}\) relationship of resistance could be seen in simulations of conventional dosing regimens but not continuous infusions (C\(_{\text{max}}\)-to-trough ratios of 6.6 and unity, respectively). It is possible that these data cannot be combined because the enrichment of the resistant mutants might depend on the shape of the simulated
pharmacokinetic profile. In our study that simulated normal and impaired gatifloxacin elimination, more pronounced loss in susceptibility of *S. aureus* was observed when concentrations oscillated significantly (C\text{max}/trough ratio of 10.8) compared with less oscillating concentrations (C\text{max}/trough ratio of 1.7). On the other hand, the simulated constant ciprofloxacin concentrations were so close to the MIC (antibiotic concentrations exceeded the MICs by a factor of 1.2) that the actual T\text{MSW} might better be described equal to zero rather than 100%. Another study that also declared the inability of T\text{MSW} to predict the selection of resistant

![Figure 7](image-url)  
**Figure 7.** AUC\text{24}/MIC relationships of resistance fitted by Equation 2: the population analysis (two upper panels) and susceptibility testing (bottom panel). Daptomycin against *S. aureus* 866 (squares) and *S. aureus* 10 (triangles); vancomycin against *S. aureus* 866 (diamonds) and *S. aureus* 10 (inverted triangles).

![Figure 8](image-url)  
**Figure 8.** T\text{MSW} relationships of resistance fitted by Equation 1 (combined data with two antibiotics and two organisms): the population analysis (at 2× MIC, top panel) and susceptibility testing (bottom panel). Daptomycin against *S. aureus* 866 (squares) and *S. aureus* 10 (triangles); vancomycin against *S. aureus* 866 (diamonds) and *S. aureus* 10 (inverted triangles).

![Figure 9](image-url)  
**Figure 9.** AUC\text{24}/MPC analysis of susceptibility data. Daptomycin against *S. aureus* 866 (squares) and *S. aureus* 10 (triangles); vancomycin against *S. aureus* 866 (diamonds) and *S. aureus* 10 (inverted triangles).
Daptomycin pharmacodynamics with staphylococci

staphylococci based their conclusions on single bolus dose or infusion of ciprofloxacin. In this design the true TnMSW relationships with resistance may not be demonstrated. Further studies that simulate multiple-dose pharmacokinetics are needed to better examine TnMSW and other potential in vitro predictors of resistant mutant selection.

AUC24/MIC relationships of the I_E and Nmax/N_initial or MIC_fine/MIC_initial ratios established in this study predict similar effects of clinical doses of both antibiotics and a greater ability of daptomycin to prevent the selection of resistant staphylococci relative to vancomycin. However, these predictions ignore the different protein binding of daptomycin (92%)9 and vancomycin (from 8% to 70%, average 42%17 and from 20% to 80%, average 55%).18 It would seem that this factor could be easily accounted for if clinically achievable AUC24/MIC90 ratios that correspond to total concentrations (380 h for 4 mg/kg daptomycin, 570 h for 6 mg/kg daptomycin and 200 h for 2× 1 g vancomycin) were simply replaced by the respective ratios calculated by multiplying AUC24/MIC90 by the free fractions determined in equilibrium dialysis or ultrafiltration studies. Assuming daptomycin binding of 92%9 and vancomycin binding of 42%,17 the respective AUC24/free/MIC90 in this scenario would be as low as 30–45 h and 116 h. As a result, the predicted effects of daptomycin on susceptible and resistant sub-populations would be much less than those based on total concentrations.

However, recent studies that simulate antibiotic concentrations with and without albumin or blood serum19–23 raise questions of whether the described method to consider protein-binding effects is correct. For example, in an in vitro study with seven differentially bound quinolones in a static in vitro study.24 In another static study that exposed the same bacterial species plus Klebsiella pneumoniae to 95% bound etrapenem and faropenem,21 their antibacterial activity was dramatically decreased in the presence of 50% human serum. These effects were observed at low but not at higher antibiotic concentrations, which, at least with S. aureus, were still much lower than clinically achievable values making the clinical relevance of these reported effects unclear. In addition, another study reported only minor effects of bovine albumin on the killing of S. pneumoniae and E. coli exposed to constant concentrations of 95–98% bound ceftiraxone.26 Recently, only minimal, if any, differences in the antistaphylococcal effects of 94%-bound telavancin calculated from reported percentages of protein binding was disappointing: pronounced reductions in bacterial titres were observed at ‘free’ concentrations below the MIC throughout the entire dosing interval (time above MIC of zero).32

As for in vitro studies that examine bacterial killing kinetics in the presence of serum, albumin and/or other macromolecules, the definitive methodology is still to be established. Until then, incorrect considerations of protein-binding effects might be even more dangerous than ignoring this factor.

Turning back to the primary goal of this study, we can conclude that data obtained with daptomycin- and vancomycin-exposed S. aureus are in support of the MSW hypothesis. Also, this study suggests that an antibiotic- and bacterial strain-independent relationship exists between an integral index of the concentration-based AUC24/MIC relationships of bacterial killing and the pharmacodynamics of antibiotics using reported percentages of protein binding are inappropriate and fraught with serious underestimation of the true antimicrobial activity, both in vitro and in vivo. For example, an attempt in infected neutropenic mice to relate the antistaphylococcal effects of free concentrations of telavancin calculated from reported percentages of protein binding was disappointing: pronounced reductions in bacterial titres were observed at ‘free’ concentrations below the MIC throughout the entire dosing interval (time above MIC of zero).32

Taken together these findings suggest that attempts to interpret the pharmacodynamics of antibiotics using reported percentages of protein binding are inappropriate and fraught with serious underestimation of the true antimicrobial activity, both in vitro and in vivo. For example, an attempt in infected neutropenic mice to relate the antistaphylococcal effects of free concentrations of telavancin calculated from reported percentages of protein binding was disappointing: pronounced reductions in bacterial titres were observed at ‘free’ concentrations below the MIC throughout the entire dosing interval (time above MIC of zero).32

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Transparency declarations

None to declare.

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