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► **To cite this version:**

M. Torrico, M.J. Giménez, N. González, L. Alou, D. Sevillano, et al.. Bactericidal activity of daptomycin versus vancomycin in the presence of human albumin against vancomycin-susceptible but tolerant meticillin-resistant (MRSA) with daptomycin minimum inhibitory concentrations of 1–2 $\mu$ g/mL. *International Journal of Antimicrobial Agents*, 2009, 35 (2), pp.131. 10.1016/j.ijantimicag.2009.09.021 . hal-00556371

**HAL Id: hal-00556371**

**<https://hal.science/hal-00556371>**

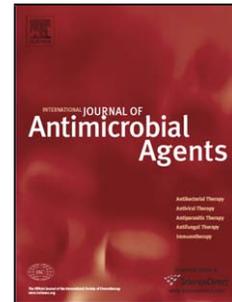
Submitted on 16 Jan 2011

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## Accepted Manuscript

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Authors: M. Torrico, M.J. Giménez, N. González, L. Alou, D. Sevillano, F. Cafini, J. Prieto, R. Cleeland, L. Aguilar

PII: S0924-8579(09)00455-5  
DOI: doi:10.1016/j.ijantimicag.2009.09.021  
Reference: ANTAGE 3146

To appear in: *International Journal of Antimicrobial Agents*

Received date: 10-9-2009  
Accepted date: 11-9-2009

Please cite this article as: Torrico M, Giménez MJ, González N, Alou L, Sevillano D, Cafini F, Prieto J, Cleeland R, Aguilar L, Bactericidal activity of daptomycin versus vancomycin in the presence of human albumin against vancomycin-susceptible but tolerant meticillin-resistant *Staphylococcus aureus* (MRSA) with daptomycin minimum inhibitory concentrations of 1–2 µg/mL, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2009.09.021

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**Bactericidal activity of daptomycin versus vancomycin in the presence of human albumin against vancomycin-susceptible but tolerant meticillin-resistant *Staphylococcus aureus* (MRSA) with daptomycin minimum inhibitory concentrations of 1–2  $\mu\text{g/mL}$**

M. Torrico <sup>a</sup>, M.J. Giménez <sup>a</sup>, N. González <sup>a</sup>, L. Alou <sup>a</sup>, D. Sevillano <sup>a</sup>, F. Cafini <sup>a</sup>, J. Prieto <sup>a</sup>, R. Cleeland <sup>b</sup>, L. Aguilar <sup>a,\*</sup>

<sup>a</sup> *Microbiology Department, School of Medicine, Universidad Complutense, Avda. Complutense s/n, 28040 Madrid, Spain*

<sup>b</sup> *Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA*

ARTICLE INFO

*Article history:*

Received 10 September 2009

Accepted 11 September 2009

*Keywords:*

MRSA

Vancomycin tolerance

Lipopeptides

Killing curves

\* Corresponding author. Tel.: +34 913 941 505; fax: +34 913 941 511.

*E-mail address:* laquilar@med.ucm.es (L. Aguilar).

## ABSTRACT

This study explored the influence of vancomycin tolerance and protein binding on the bactericidal activity of vancomycin versus daptomycin (protein binding 36.9% vs. 91.7%, respectively) against four vancomycin-tolerant methicillin-resistant *Staphylococcus aureus* (MRSA) [minimum inhibitory concentration/minimum bactericidal concentration (MIC/MBC) = 0.5/16, 1/32, 2/32 and 1/32  $\mu\text{g/mL}$  for vancomycin and 1/1, 1/2, 2/2 and 2/4  $\mu\text{g/mL}$  for daptomycin]. Killing curves were performed with vancomycin/daptomycin concentrations equal to serum peak concentrations ( $C_{\text{max}}$ ) (65.70/98.60  $\mu\text{g/mL}$ ) and trough concentrations ( $C_{\text{min}}$ ) (7.90/9.13  $\mu\text{g/mL}$ ) in the presence and absence of a physiological human albumin concentration (4 g/dL), controlled with curves with the theoretical free drug fraction of vancomycin/daptomycin  $C_{\text{max}}$  (41.45/8.18  $\mu\text{g/mL}$ ) and  $C_{\text{min}}$  (4.98/0.76  $\mu\text{g/mL}$ ). Vancomycin  $C_{\text{max}}$  and  $C_{\text{min}}$  concentrations, regardless of the media, showed a bacteriostatic profile not reaching a reduction of 99% or 99.9% of the initial inocula during the 24-h experimental time period. Daptomycin antibacterial profiles significantly differed when testing  $C_{\text{max}}$  and  $C_{\text{min}}$ .  $C_{\text{max}}$  was rapidly bactericidal ( $\leq 4$  h) with  $>5 \log_{10}$  reduction in the initial inocula for all strains, regardless of the presence or not of albumin or the use of concentrations similar to free  $C_{\text{max}}$ .  $C_{\text{min}}$  exhibited similar final colony counts at 0 h and 24 h in curves with albumin, but with  $>3 \log$  colony-forming units (CFU)/mL reduction at  $\leq 4$  h for strains with an MIC of 1  $\mu\text{g/mL}$  and ca. 2 log CFU/mL reduction at  $\leq 6$  h for strains with an MIC of 2  $\mu\text{g/mL}$ . This activity was significantly higher than the activity of the free  $C_{\text{min}}$  fraction. The results of this study reinforce the idea that pharmacodynamics using concentrations calculated using reported protein binding are unreliable. Daptomycin exhibited rapid antibacterial activity against vancomycin-tolerant MRSA isolates even against those with high daptomycin MICs in the presence of physiological albumin concentrations.

## 1. Introduction

Following the introduction of penicillin, staphylococci as a cause of infection could be treated, however soon after resistance due to  $\beta$ -lactamase production became prevalent among *Staphylococcus aureus*. As a consequence, isoxazolyl penicillins were introduced to the market with the consequent emergence of meticillin resistance in *S. aureus*. Nowadays, meticillin resistance in Spain is ca. 30% as reported in a transverse prevalence study including 143 hospitals [1]. Following the emergence of meticillin resistance, meticillin-resistant *S. aureus* (MRSA) strains responded to vancomycin pressure by a series of metabolic changes driving different vancomycin resistance phenotypes and tolerance [2]. The most frequent resistance phenotype is the heterogeneous vancomycin-intermediate-resistant *S. aureus* (hVISA), ranging from 0% to 50% in different studies, followed by homoresistant vancomycin-intermediate *S. aureus* (VISA) (10% of all vancomycin-intermediate-resistant isolates) and with inappreciable spread of vancomycin-resistant *S. aureus* (VRSA) strains [2–7]. Vancomycin tolerance is defined as a minimum bactericidal concentration:minimum inhibitory concentration (MBC:MIC) ratio of  $\geq 32$  or an MBC:MIC ratio  $\geq 16$  with an MBC value in the resistant range [8,9] and it can be assumed that 100% of VISA and VRSA, 74% of hVISA and 15% of wild-type MRSA are tolerant to this antibiotic [8]. Tolerance may have clinical implications as reflected in small studies and clinical case reports [2,10–13] and its development in susceptible isolates is significantly associated with a reduction in favourable clinical responses in the treatment of *S. aureus* bacteraemia [14].

The possibility of vancomycin resistance among MRSA has led to the development of new agents such as the lipopeptide daptomycin with greater bactericidal activity despite its high protein binding [15].

This study explored the in vitro bactericidal activity of daptomycin versus vancomycin using concentrations similar to peak ( $C_{max}$ ) and trough ( $C_{min}$ ) serum concentrations achieved in humans following standard doses in the presence and absence of physiological human albumin concentrations against vancomycin-tolerant MRSA strains with high daptomycin MICs.

## 2. Materials and methods

### 2.1. Strains

Four vancomycin-tolerant MRSA clinical isolates with cloxacillin MICs of 4  $\mu\text{g}/\text{mL}$  for strains 1, 2 and 3 and 128  $\mu\text{g}/\text{mL}$  for strain 4 were used throughout the study. MICs and MBCs of daptomycin and vancomycin were determined five times following Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards) recommendations [16,17] with a final inoculum of  $5 \times 10^5$  colony-forming units (CFU/mL) and modal values were considered. The test medium was cation-adjusted Mueller–Hinton broth (MHB) supplemented with calcium to a target level of 50  $\mu\text{g}/\text{mL}$  (pH 7.2) as suggested by the CLSI [16]. Vancomycin tolerance was defined as an MBC:MIC ratio of  $\geq 16$ .

## 2.2. Killing curves

Killing curves were performed with final inocula ranging from  $1 \times 10^7$  CFU/mL to  $5 \times 10^7$  CFU/mL and final concentrations of 98.60  $\mu\text{g/mL}$  ( $C_{\text{max}}$ ) or 9.13  $\mu\text{g/mL}$  ( $C_{\text{min}}$ ) for daptomycin and of 65.70  $\mu\text{g/mL}$  ( $C_{\text{max}}$ ) or 7.90  $\mu\text{g/mL}$  ( $C_{\text{min}}$ ) for vancomycin, corresponding to steady-state peak and trough concentrations in serum following an intravenous dose of 6 mg/kg daptomycin and 1 g of vancomycin [18,19]. Two different media were used: MHB (Difco, Detroit, MI); and MHB with 4 g/dL human albumin (A-1653; Sigma-Aldrich). In parallel, killing curves with concentrations corresponding to free drug  $C_{\text{max}}$  and  $C_{\text{min}}$  when considering protein binding (91.7% for daptomycin [18] and 36.9% for vancomycin [20]) were performed in MHB: 8.18  $\mu\text{g/mL}$  (free  $C_{\text{max}}$ ) and 0.76  $\mu\text{g/mL}$  (free  $C_{\text{min}}$ ) for daptomycin and 41.45  $\mu\text{g/mL}$  (free  $C_{\text{max}}$ ) and 4.98  $\mu\text{g/mL}$  (free  $C_{\text{min}}$ ) for vancomycin. MHB was supplemented with 50 mg/L  $\text{Ca}^{2+}$  according to CLSI recommendations for daptomycin [16], except in tubes with human albumin where 100 mg/L  $\text{Ca}^{2+}$  was added to obtain a physiological free  $\text{Ca}^{2+}$  concentration [21]. Antibiotic-free control growth curves were performed in MHB with and without albumin. Samples for colony counting were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h. To prevent carry-over, 1:100 dilutions were performed (maximal residual concentration of daptomycin or vancomycin below the MIC of the microorganisms tested), or by vacuum filtration through 0.45  $\mu\text{m}$  membranes (S-Pak membrane filters; Millipore, Molsheim, France) when the estimated colony counts were lower than the limit of detection of the dilution method. The resulting samples were plated onto Mueller-Hinton agar supplemented with 5% sheep blood (Biomedics, Madrid, Spain) and were incubated at 37 °C for 24 h. Colony counts were performed using a spiral platter (Don Whitley, Shipley, UK). All experiments were performed in triplicate. The limit of detection was 20 CFU/mL.

### 2.3. Statistical analysis

Log<sub>10</sub> reductions (log<sub>10</sub> colony counts at time 0 – log<sub>10</sub> colony counts at each sampling time) were calculated.  $E_{\max}$  was defined as the maximum log<sub>10</sub> reduction. Times to obtain 90%, 99% and 99.9% reductions in initial inocula (1, 2 and 3 log<sub>10</sub> reductions, respectively) were determined ( $T_{90}$ ,  $T_{99}$  and  $T_{99.9}$ , respectively). Comparisons between log<sub>10</sub> reductions were performed by analysis of variance (ANOVA) with the Tukey's test for multiple comparisons. Owing to multiple comparisons, a  $P < 0.001$  was considered statistically significant.

## 3. Results

MIC/MBC values of vancomycin for the study strains were 0.5/16, 1/32, 2/32 and 1/32 µg/mL for strains 1, 2, 3 and 4, respectively (Tables 1 and 2). All strains were vancomycin-susceptible (susceptibility breakpoint  $\leq 2$  µg/mL) [22] but tolerant, with MBC:MIC ratios of 32 for strains 1, 2 and 4 and of 16 for strain 3. With respect to daptomycin, two strains were susceptible (susceptibility breakpoint  $\leq 1$  µg/mL) [22] with MICs of 1 µg/mL, and two were non-susceptible with MICs of 2 µg/mL.

For all strains, initial inocula ranged from 7.25 to 7.35 log<sub>10</sub> CFU/mL in all experimental curves. Final colony counts in control curves (media without antibiotics) ranged from 8.58 to 8.78 log<sub>10</sub> CFU/mL with no differences in controls with and without albumin (Figs 1 and 2).

Table 1 shows the  $T_{90}$ ,  $T_{99}$ ,  $T_{99.9}$  and  $E_{\max}$  values provided by daptomycin and vancomycin against daptomycin-susceptible strains, and Table 2 shows the same parameters for daptomycin non-susceptible strains.

All the antibacterial profiles obtained with vancomycin were similar regardless of testing  $C_{\max}$  (Fig. 1) or  $C_{\min}$  (Fig. 2), total concentrations (in the presence or absence of albumin) or concentrations similar to free drug fractions.  $T_{99}$  and  $T_{99.9}$  was  $>24$  h (i.e. 99% and 99.9% reductions in initial inocula were not achieved in the experimental time) since  $E_{\max}$  was  $<2 \log_{10}$  for the four strains regardless of the concentration tested or the composition of the media.  $T_{90}$  was 24 h in curves with  $C_{\max}$  or  $C_{\min}$  in the presence of albumin for strains 1, 2 and 4, but not for strain 3 [the strain with the highest vancomycin MIC ( $2 \mu\text{g/mL}$ )]. In all killing curves with vancomycin, mean  $\log_{10}$  colony counts at 24 h were  $\geq 5.57$  (Figs 1 and 2).

The antibacterial profiles of daptomycin significantly differed when testing  $C_{\max}$  (Fig. 1) and  $C_{\min}$  (Fig. 2). With respect to  $C_{\max}$ , for all study strains negligible differences were found between curves in media with albumin, those in broth without albumin and those with concentrations similar to the free drug fraction of  $C_{\max}$  (Fig. 1).  $T_{99.9}$  was  $\leq 4$  h and mean  $E_{\max}$  was  $>5 \log_{10}$  for all study strains [whether daptomycin-susceptible (Table 1) or non-susceptible with daptomycin MIC of  $2 \mu\text{g/mL}$  (Table 2)], regardless of the presence or not of albumin in the media or the use of concentrations similar to the free drug fraction of daptomycin  $C_{\max}$ . Colony counts at 24 h were  $\leq 2.2 \log_{10}$  CFU/mL in all  $C_{\max}$  killing curves.

With respect to daptomycin  $C_{min}$ ,  $E_{max}$  was higher in the curves with the  $C_{min}$  in broth than in those performed in broth with albumin, reaching statistical significance ( $P < 0.001$ ) for strains 1, 3 and 4 (with identical  $E_{max}$  in the case of strain 2).  $E_{max}$  in the curves performed with  $C_{min}$  in the presence of human albumin was higher than in those performed with concentrations similar to the free drug fraction of  $C_{min}$  (Fig. 1), reaching statistical significance ( $P < 0.001$ ) for strains 1, 2 and 4 but not for strain 3 ( $P = 0.01$ ) (Tables 1 and 2). For all strains,  $T_{99}$  was 2 h in curves with  $C_{min}$  in broth,  $\leq 6$  h in curves with  $C_{min}$  in the presence of albumin and  $>24$  h in curves with concentrations similar to the free drug fraction of  $C_{min}$ . Colony counts at 24 h were  $\leq 3.3 \log_{10}$  CFU/mL in curves with broth without albumin and were 5.4 and 6.4  $\log_{10}$  CFU/mL for strains 1 and 2, respectively, and similar to initial inocula for strains 3 and 4 in curves with broth and albumin, whilst in curves with concentrations similar to free drug fraction of the  $C_{min}$  colony counts increased similarly as in control curves.

Significantly higher  $E_{max}$  and  $\log_{10}$  reductions at 24 h were obtained with daptomycin versus vancomycin in all  $C_{max}$  curves for all strains. With respect to  $C_{min}$  curves, significantly ( $P < 0.001$ ) higher  $E_{max}$  was obtained with daptomycin versus vancomycin in  $C_{min}$  curves in broth for all strains, in  $C_{min}$  curves in the presence of albumin for daptomycin-susceptible strains [not reaching statistical significance for strains 3 ( $P = 0.36$ ) and 4 ( $P = 0.003$ )], but without differences between daptomycin and vancomycin in curves with concentrations similar to the free drug fraction of  $C_{min}$ .

#### 4. Discussion

It is generally assumed that due to its unique mechanism of action daptomycin non-susceptibility is very difficult to generate. In this regard, in a 5-year study in Spain MIC

values of daptomycin in methicillin-susceptible *S. aureus* (MSSA) and MRSA were practically unaltered with no detection of non-susceptible strains [23]. It has been suggested that the thickened wall of vancomycin-intermediate isolates, as a barrier for the penetration of vancomycin molecules, may also act as a barrier for daptomycin [24]. However, daptomycin non-susceptibility has been found not only among a unique set of vancomycin-resistant isolates at the US Centers for Disease Control and Prevention (CDC) [25] but also among vancomycin-susceptible isolates [26]. Nevertheless, daptomycin non-susceptibility is exceptional, being <0.1% during 2002–2005 in North American hospitals [26].

In a previous *in vitro* study, vancomycin concentrations similar to the  $C_{max}$  exhibited slow activity, being bactericidal (i.e. producing  $\geq 3 \log_{10}$  reduction in the initial inocula) only at 24 h against vancomycin-susceptible and non-tolerant MSSA and MRSA, and non-bactericidal against the non-tolerant hVISA [15]. Those strains were daptomycin-susceptible and this antibiotic was rapidly bactericidal (2 h for MRSA and 4 h for hVISA) even in the presence of physiological concentrations of human albumin [15]. Since tolerance can preclude bactericidal activity, and this may have clinical implications [27], the question of whether daptomycin can counter vancomycin tolerance not only in daptomycin-susceptible but also in the rare daptomycin non-susceptible isolates arises.

In the present study, we used four vancomycin-susceptible but tolerant MRSA, two of them susceptible (MIC = 1  $\mu\text{g}/\text{mL}$ ) and two non-susceptible (MIC = 2  $\mu\text{g}/\text{mL}$ ) to daptomycin. Bactericidal activity was measured in the presence and absence of physiological concentrations of human albumin to assess the effect of protein binding, especially important in the case of daptomycin that is highly bound (91.7%) in

comparison with vancomycin (36.9%) [18,20], and compared it with the activity obtained with concentrations similar to free drug fractions calculated using reported protein binding. In the present study we also tested  $C_{\min}$  concentrations (trough concentrations measured prior to administration of the next dose) since in a previous study the high activity displayed by daptomycin  $C_{\max}$  concentrations could negatively affect a clear evaluation of the protein binding effect. The use of  $C_{\max}$  and  $C_{\min}$  concentrations in this static experiment (killing curves) is a clear limitation of the study since they do not represent the in vivo situation with concentrations changing over time. However, they allow an assessment of the effect of protein binding on the antimicrobial activity of a compound in the most favourable ( $C_{\max}$ ) and worst ( $C_{\min}$ ) conditions.

In this context, the slow bactericidal activity of vancomycin  $C_{\max}$  shown against non-tolerant MRSA in a previous study [15] was precluded by the presence of MBC:MIC ratios  $\geq 16$  in the four strains in the present study. Vancomycin  $C_{\max}$  and  $C_{\min}$  showed a bacteriostatic profile never reaching a reduction of 99% or 99.9% of the initial inocula during the experimental time ( $T_{99}$  and  $T_{99.9} > 24$  h, a time longer than two dosing intervals).

With respect to daptomycin, as in the previous study, the high bactericidal activity displayed by  $C_{\max}$  even against daptomycin non-susceptible strains (MIC = 2  $\mu\text{g}/\text{mL}$ ) precluded a clear assessment of the effect of protein binding, since  $T_{99.9}$  was  $\leq 4$  h for all strains regardless of the presence or not of albumin or the use of concentrations similar to the free drug fraction. As expected, this could be clearly assessed with  $C_{\min}$  since the profile obtained with daptomycin concentrations similar to the free  $C_{\min}$  fraction was identical to antibiotic-free controls for the four strains, and logically its  $E_{\max}$  was

significantly lower than the  $E_{\max}$  obtained in curves with  $C_{\min}$  in the presence of albumin, again for the four isolates whether or not susceptible to daptomycin. Daptomycin  $C_{\min}$  exhibited a bacteriostatic profile (similar final colony counts at 0 h and 24 h) in curves with physiological albumin concentrations, but with an  $E_{\max} > 3 \log \text{CFU/mL}$  obtained at  $\leq 4$  h for susceptible strains ( $T_{99.9} \leq 4$  h) and an  $E_{\max}$  of ca.  $2 \log \text{CFU/mL}$  obtained at  $\leq 6$  h for non-susceptible strains ( $T_{99.9} \leq 6$  h), time periods less than one-half of the dosing interval of daptomycin. The re-growths observed with  $C_{\min}$  concentrations in the presence of albumin from 6–8 h onwards (Fig. 2) were a consequence of the abovementioned study design, not having translation to the in vivo situation where the bacterial mass is continuously exposed to changing antibiotic concentrations that are always higher than trough concentrations which only are present just immediately prior to administration of the next dose. In the face of this, the achievement of significant  $E_{\max}$  values ( $\geq 2 \log_{10}$  reductions in the initial inocula) with  $C_{\min}$  in the presence of albumin could be demonstrative of the antibacterial activity (bactericidal at most time points based on the  $C_{\max}$  and  $C_{\min}$   $E_{\max}$  values in this study) of all concentrations of daptomycin obtained during the dosing interval. In contrast, the bacteriostatic profile of  $C_{\max}$  and  $C_{\min}$  of vancomycin, regardless or not of the presence of albumin, implies the absence of bactericidal activity throughout the dosing interval against tolerant strains. In this sense, studies exploring continuous exposure simulating serum profiles in pharmacodynamic computerised devices are warranted to explore further the tolerance phenomenon in *S. aureus*, as has been done previously in *Enterococcus faecium* [28].

Whilst some authors have suggested that complicated infections caused by vancomycin-tolerant strains may be more difficult to treat [14,27,29], others have doubt about the need for bactericidal activity for the treatment of *S. aureus* infections [30,31].

However, classical studies suggest the need for bactericidal activity. In this sense, a study carried out in Spain in patients with bacteraemia due to MSSA showed significantly higher mortality in vancomycin-treated versus cloxacillin-treated patients [32]. If the compromise of bactericidal activity by tolerance has clinical implications, then the magnitude of the tolerance problem should be measured. Whilst some studies set at 15% vancomycin tolerance in wild-type MRSA [8], a recent study reported rates of 18%, 6.1% and 0% to teicoplanin, vancomycin and daptomycin, respectively [9].

The results of this study show that daptomycin  $C_{max}$  exhibited rapid bactericidal activity against vancomycin-tolerant MRSA clinical isolates susceptible or not to daptomycin, whilst daptomycin  $C_{min}$  exhibited a significant  $E_{max}$  (99.9% reduction in initial inocula for susceptible strains and 90% for non-susceptible strains) in periods shorter than the dosing interval and in the presence of physiological albumin concentrations. In relation to protein binding, the differences observed between curves with  $C_{min}$  in the presence of albumin and those with concentrations similar to the  $C_{min}$  free drug fraction reinforce the idea that attempts to interpret pharmacodynamics using reported percentages of protein binding are inappropriate and fraught with underestimations of antimicrobial activity.

### **Acknowledgment**

Part of this study was presented at the 19th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 16–19 May 2009, Helsinki, Finland.

### **Funding**

This study was supported in part by an unrestricted grant from Novartis Pharma AG (Basel, Switzerland).

**Competing interests**

RC is an employee of Novartis Pharmaceuticals Corporation, USA. The other authors declare no competing interests.

**Ethical approval**

Not required.

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**Fig. 1.** Mean  $\pm$  standard deviation bacterial counts [ $\log_{10}$  colony-forming units (CFU)/mL] over 24 h in curves with antibiotic-free broth (control; black dotted lines with open circles), antibiotic-free broth with albumin (control; grey dotted lines with open circles), peak serum concentration ( $C_{\max}$ ) in broth (black continuous lines with filled circles) and  $C_{\max}$  in broth with albumin (grey continuous lines with filled circles) and in curves with concentrations corresponding to free drug  $C_{\max}$  in broth (black dotted lines with filled circles) for strains 1, 2, 3 and 4 for daptomycin (left column) and vancomycin (right column).

**Fig. 2.** Mean  $\pm$  standard deviation bacterial counts [ $\log_{10}$  colony-forming units (CFU)/mL] over 24 h in curves with antibiotic-free broth (control; black dotted lines with open circles), antibiotic-free broth with albumin (control; grey dotted lines with open circles), trough serum concentration ( $C_{\min}$ ) in broth (black continuous lines with filled circles) and  $C_{\min}$  in broth with albumin (grey continuous lines with filled circles) and in curves with concentrations corresponding to free drug  $C_{\min}$  in broth (black dotted lines with filled circles) for strains 1, 2, 3 and 4 for daptomycin (left column) and vancomycin (right column).

**Table 1**

Time (h) to obtain 90% (1 log<sub>10</sub>), 99% (2 log<sub>10</sub>) and 99.9% (≥3 log<sub>10</sub>) reduction of initial inocula ( $T_{90}$ ,  $T_{99}$  and  $T_{99.9}$ , respectively) and maximal effect ( $E_{max}$ ) (mean log<sub>10</sub> ± standard deviation) against daptomycin-susceptible strains

	Daptomycin				Vancomycin			
	$T_{90}$	$T_{99}$	$T_{99.9}$	$E_{max}$	$T_{90}$	$T_{99}$	$T_{99.9}$	$E_{max}$
Strain 1	MIC = 1 µg/mL; MBC = 1 µg/mL				MIC = 0.5 µg/mL; MBC = 16 µg/mL			
$C_{max}$	1	2	2	5.9 ± 0.0	>24	>24	>24	0.5 ± 0.3
$C_{max}$ -Alb	2	2	4	6.0 ± 0.1	>24	>24	>24	0.9 ± 0.5
Free $C_{max}$	2	2	3	5.1 ± 0.6	>24	>24	>24	0.5 ± 0.4
$C_{min}$	2	2	4	4.9 ± 0.4	>24	>24	>24	0.3 ± 0.3
$C_{min}$ -Alb	2	3	4	3.6 ± 0.3	>24	>24	>24	0.8 ± 0.4
Free $C_{min}$	3	>24	>24	1.1 ± 0.3	>24	>24	>24	0.4 ± 0.6
Strain 2	MIC = 1 µg/mL; MBC = 2 µg/mL				MIC = 1 µg/mL; MBC = 32 µg/mL			
$C_{max}$	1	1	1	6.0 ± 0.2	24	>24	>24	1.2 ± 0.3
$C_{max}$ -Alb	1	2	2	6.0 ± 0.2	24	>24	>24	1.8 ± 0.3
Free $C_{max}$	1	2	2	6.0 ± 0.2	24	>24	>24	1.5 ± 0.2
$C_{min}$	1	2	2	6.0 ± 0.2	>24	>24	>24	0.4 ± 0.2
$C_{min}$ -Alb	2	3	4	6.0 ± 0.2	24	>24	>24	1.2 ± 0.2
Free $C_{min}$	>24	>24	>24	0.6 ± 0.4	>24	>24	>24	0.7 ± 0.2

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration;

$C_{max/min}$ , serum peak/trough concentration;  $C_{max/min}$ -Alb, serum peak/trough concentration

with albumin; Free  $C_{max/min}$ , free drug fraction of  $C_{max/min}$ .

**Table 2**

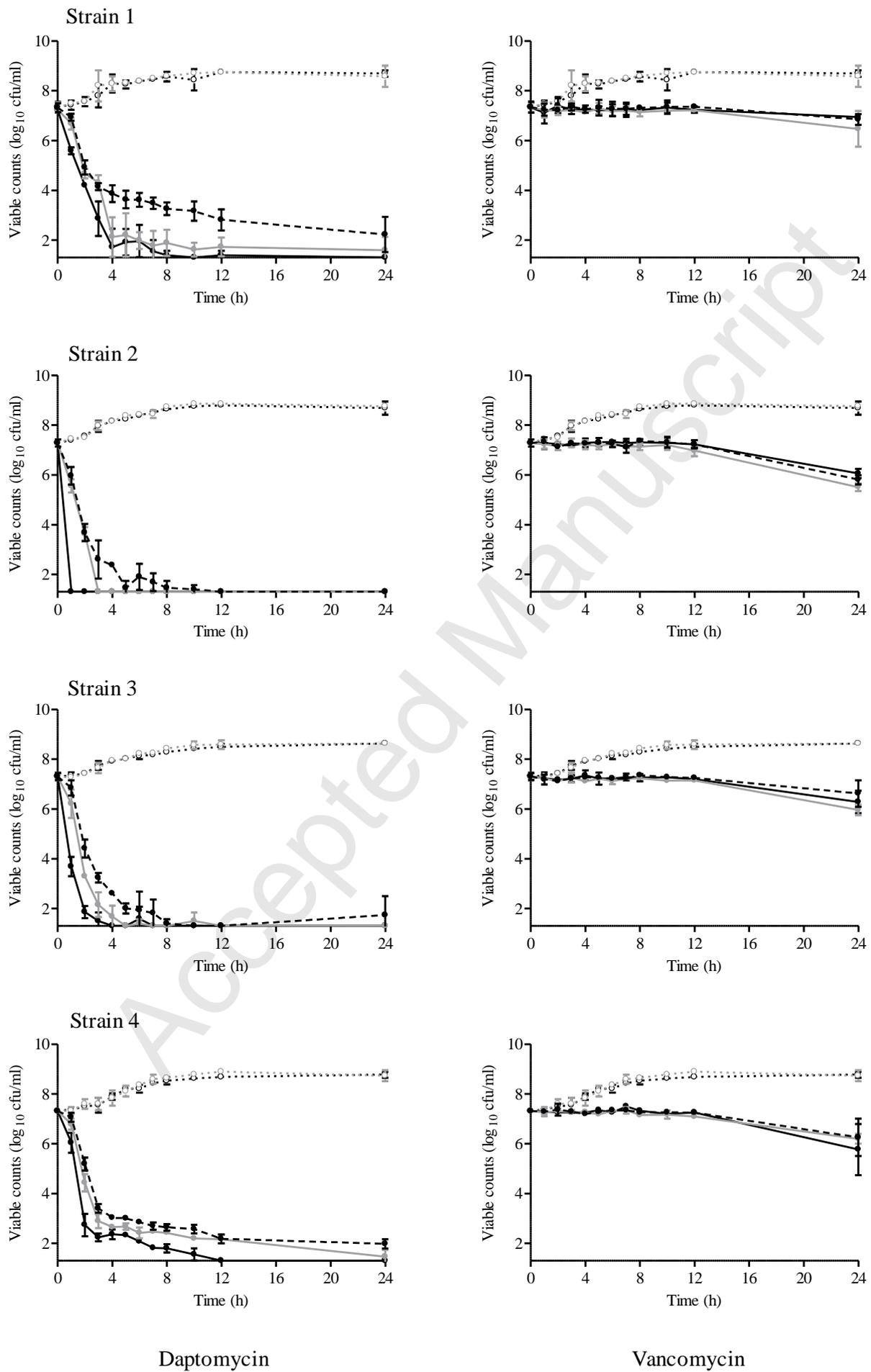
Time (h) to obtain 90% (1 log<sub>10</sub>), 99% (2 log<sub>10</sub>) and 99.9% (≥3 log<sub>10</sub>) reduction of initial inocula ( $T_{90}$ ,  $T_{99}$  and  $T_{99.9}$ , respectively) and maximal effect ( $E_{max}$ ) (mean log<sub>10</sub> ± standard deviation) against daptomycin-non susceptible strains

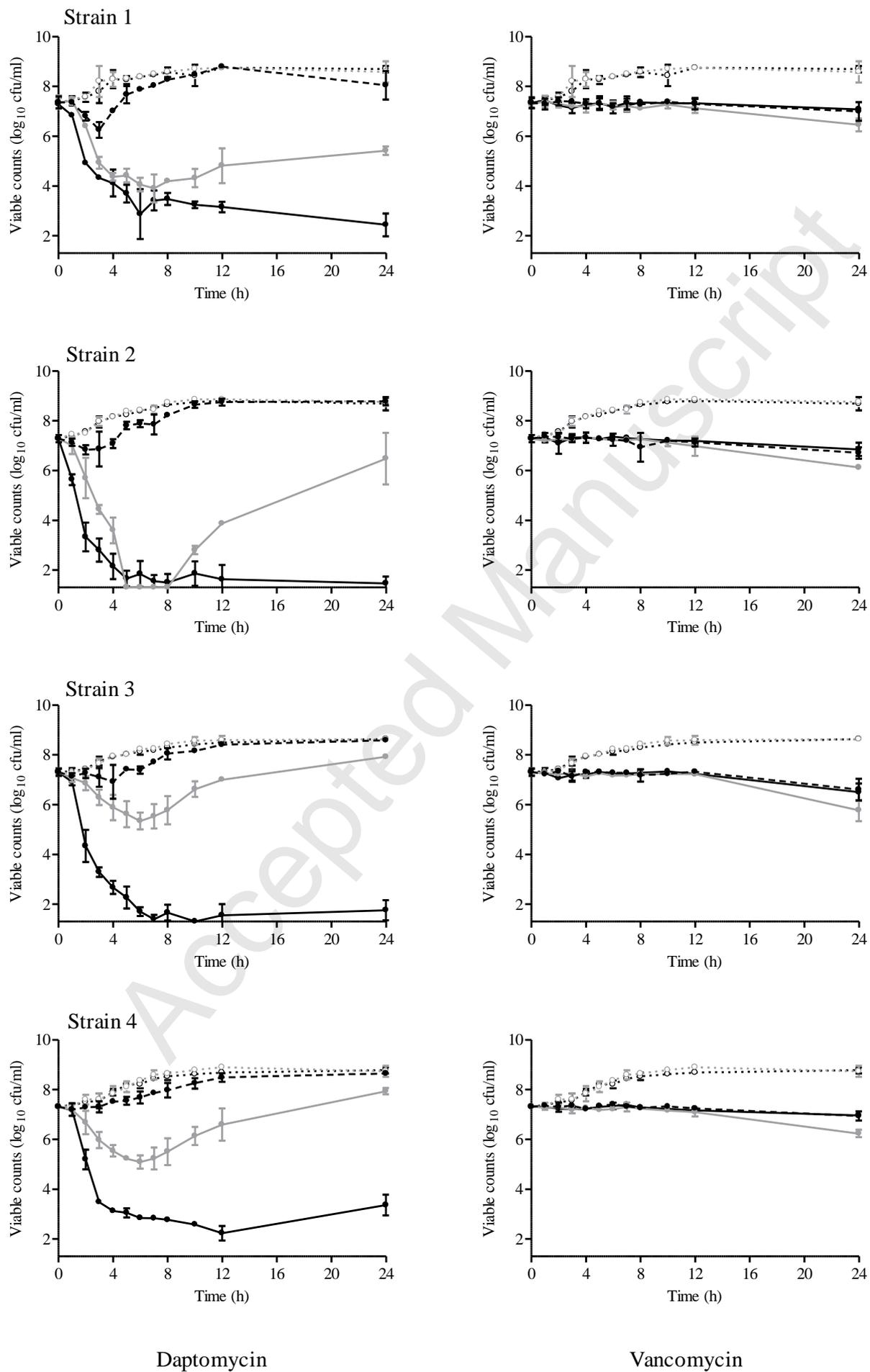
	Daptomycin				Vancomycin			
	$T_{90}$	$T_{99}$	$T_{99.9}$	$E_{max}$	$T_{90}$	$T_{99}$	$T_{99.9}$	$E_{max}$
Strain 3	MIC = 2 µg/mL; MBC = 2 µg/mL				MIC = 2 µg/mL; MBC = 32 µg/mL			
$C_{max}$	1	1	1	6.0 ± 0.1	24	>24	>24	1.0 ± 0.6
$C_{max}$ -Alb	1	2	2	6.0 ± 0.1	24	>24	>24	1.3 ± 0.4
Free $C_{max}$	2	2	3	6.0 ± 0.1	>24	>24	>24	0.7 ± 0.6
$C_{min}$	2	2	3	6.0 ± 0.1	>24	>24	>24	0.8 ± 0.5
$C_{min}$ -Alb	3	6	>24	2.0 ± 0.5	24	>24	>24	1.5 ± 0.6
Free $C_{min}$	>24	>24	>24	0.5 ± 0.7	>24	>24	>24	0.7 ± 0.6
Strain 4	MIC = 2 µg/mL; MBC = 4 µg/mL				MIC = 1 µg/mL; MBC = 32 µg/mL			
$C_{max}$	1	2	2	6.0 ± 0.1	24	>24	>24	1.6 ± 1.0
$C_{max}$ -Alb	2	2	3	5.9 ± 0.2	24	>24	>24	1.1 ± 0.2
Free $C_{max}$	2	2	3	5.3 ± 0.1	24	>24	>24	1.1 ± 0.8
$C_{min}$	2	2	3	5.1 ± 0.3	>24	>24	>24	0.4 ± 0.1
$C_{min}$ -Alb	3	5	>24	2.2 ± 0.3	24	>24	>24	1.1 ± 0.2
Free $C_{min}$	>24	>24	>24	0.2 ± 0.2	>24	>24	>24	0.4 ± 0.2

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration;

$C_{max/min}$ , serum peak/trough concentration;  $C_{max/min}$ -Alb, serum peak/trough concentration

with albumin; Free  $C_{max/min}$ , free drug fraction of  $C_{max/min}$ .





Daptomycin

Vancomycin