Re-Evaluation of the Bactericidal Activities and the Correlation between Disk Diffusion and MIC Methods for Testing Daptomycin and Vancomycin against *S. aureus* with Decreased Susceptibility (S) to Gylcopeptides

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ABSTRACT

Background: Daptomycin (DAP), a cyclic lipopeptide with broad spectrum and potent antimicrobial activity against Gram-positive pathogens including oxacillin-resistant (R) *S. aureus* (MRSA), has disk diffusion (DD) testing guidelines that have been recently challenged. We characterized the MRSA strains most likely to be problematic. **Methods:** A total of 210 *S. aureus* strains, including 105 well-characterized strains with decreased S to VAN

(88 hVISA and 17 VISA) and 105 MRSA-WT with VAN MIC \leq 2 µg/ml (isolated in 2003) were S tested by reference CLSI broth microdilution (BMD) and DD methods, as well as by Etest (ET), against DAP and VAN. The lowest concentration of antimicrobial agent that killed \geq 99.9% of the initial inoculum was defined as the MBC endpoint. Time kill-curves were performed in selected strains to confirm MBC results. **Results:** All MRSA-WT and hVISA strains were inhibited by \leq 1 µg/ml of DAP, while the VISA strains showed slightly higher DAP MIC values (range, 0.5 – 4 µg/ml). All DAP MBC results were at the MIC or two-fold greater.

slightly higher DAP MIC values (range, 0.5 – 4 µg/ml). All DAP MBC results were at the MIC or two-fold greater In contrast, 17.1% of MRSA-WT, 69.3% of hVISA and all VISA strains showed a VAN MBC/MIC at ≥ 16. The correlation coefficient (r) between BMD and DD was low for DAP (0.07) and VAN (0.11); poor tests. Eight (3.8%) strains, all in the hVISA/VISA group, were non-S to DAP by BMD but S by DD. Against VAN, 7 (3.3% strains were non-S by BMD but S by DD. ET showed a slight tendency towards lower DAP MIC but higher VAN MIC, with very major and major error rates of 1.0 and 1.0% for DAP, and 1.9 and 1.0% for VAN, respectively All discrepant results were among the hVISA/VISA isolates.

Conclusions: DAP was highly bactericidal against *S. aureus* strains and its bactericidal activity, in contrast to VAN, was not affected by reduced susceptibility to VAN. DD generally failed to detect strains categorized as non-S to DAP or VAN by BMD (hVISA/VISA), and re-assessment of breakpoints should be immediately attempted.

INTRODUCTION

Daptomycin is a cyclic lipopeptide which has been recently approved by the United States Food and Drug Administration (US-FDA) for the treatment of complicated skin and skin structure infections and has been evaluated for the treatment of several other infections, including bacterial endocarditis. Daptomycin monotherapy was shown to be superior to vancomycin monotherapy in the treatment of experimental endocarditis due to methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA), as determined by a statistically significant decrease in the bacterial concentration of aortic valve vegetations after five days of therapy.

Daptomycin has a unique mechanism of action with no cross resistance to glycopeptide (teicoplanin and vancomycin) resistant strains. Daptomycin acts on the cytoplasmic membrane in the presence of physiological levels of calcium ions. Its in vitro susceptibility testing requires appropriate supplementation of calcium to the test media (50 µg/ml in broth).

In a previous study, we evaluated the bactericidal activities of daptomycin and vancomycin against vancomycin-intermediate (VISA) and hetero-VISA *S. aureus* strains as compared to a methicillin- (oxacillin-) resistant *S. aureus* (MRSA) collection of wild type (WT) strains. In the present study, we evaluated the correlation between the susceptibility results generated by broth microdilution, disk diffusion and Etest for these two antimicrobials. We also evaluated the presence of a subpopulation with elevated vancomycin MIC values (heteroresistance [hVISA]) and the bactericidal activity of vancomycin and daptomycin by time kill curve experiments on MRSA-WT isolates with elevated MBC/MIC ratios for vancomycin.

MATERIALS AND METHODS

Organism collection: A collection of 210 strains was selected to evaluate the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for vancomycin and daptomycin, and the correlation between broth microdilution, disk diffusion and Etest results for these two antimicrobials. The collection is composed of two groups of strains:

- A. hVISA/VISA group: 105 isolates
- 1. hVISA subset: Includes 88 isolates with vancomycin MIC results \leq 4 µg/ml by reference broth microdilution method that show a subpopulation with a vancomycin MIC result > 4 µg/ml when tested with high inoculum (heterogeneous population).
- 2. VISA subset: Includes 17 isolates with vancomycin MIC results of 4 or 8 μg/ml and a homogenous population. Ten strains were characterized by methods described by Wootton et al. [2001] and seven strains were provided by the Network on Antimicrobial Resistance in *S. aureus* (NARSA; <u>www.narsa.net</u>).
- B. MRSA-WT group: 105 oxacillin-resistant *S. aureus* strains with a homogenous population with vancomycin MIC results ≤ 2 μg/ml (wild type). These isolates were collected from >50 medical centers worldwide in 2003. No more than two strains per medical center were included, one collected in January and the other collected in December.
- 1. Fifteen of these strains were selected for the kill curve experiments because they demonstrated high MBC/MIC ratios for vancomycin. These strains were also evaluated by Etest against vancomycin and daptomycin using high (2 McFarland) inoculums in order to detect subpopulations with high MIC values for vancomycin (hVISA).

Susceptibility testing: MIC values were determined by broth microdilution methods for daptomycin, vancomycin and oxacillin according to CLSI/NCCLS procedures. Appropriate medium variations (50 mg/L of calcium) were used for testing daptomycin.

Disk diffusion tests with 30-µg daptomycin and vancomycin disks were performed on all 210 isolates according to the methods recommended by the CLSI/NCCLS with 150-mm Mueller-Hinton agar plates.

MICs for vancomycin and daptomycin were also evaluated by Etest methodology on all 210 isolates according to the manufacturer's instructions. In addition, MICs for vancomycin, teicoplanin and daptomycin were evaluated by Etest methodology using standard (NCCLS, 2003) and high (2 McFarland) inoculums on 15 selected strains and two controls. Mueller-Hinton agar was used for the standard inoculum, while brain-heart infusion (BHI) agar was used for the 2 McFarland inoculum according to procedures reported by Wooton et al. (2001). This technique

was used to detect strains having hetero-resistance to vancomycin (hVISA), and the criteria used to characterize hVISA was as follow: MIC values \geq 8 µg/ml for both vancomycin and teicoplanin after 48 hours of incubation at 37°C (Wootton et al., 2001).

Minimal Bactericidal Activity: MBC values were assessed for daptomycin and vancomycin by plating all (0.1 ml) the broth from the MIC well and from the log_2 dilutions above the MIC for each organism onto appropriate growth media. Quantitative colony counts were performed on the starting inoculum at the time the MIC test was initiated. The lowest concentration of antimicrobial agent that killed $\geq 99.9\%$ of the starting inoculum was defined as the MBC endpoint. Tolerance was defined as a MBC/MIC ratio ≥ 32 or ≥ 16 with an associated MBC at ≥ 16 µg/ml for vancomycin.

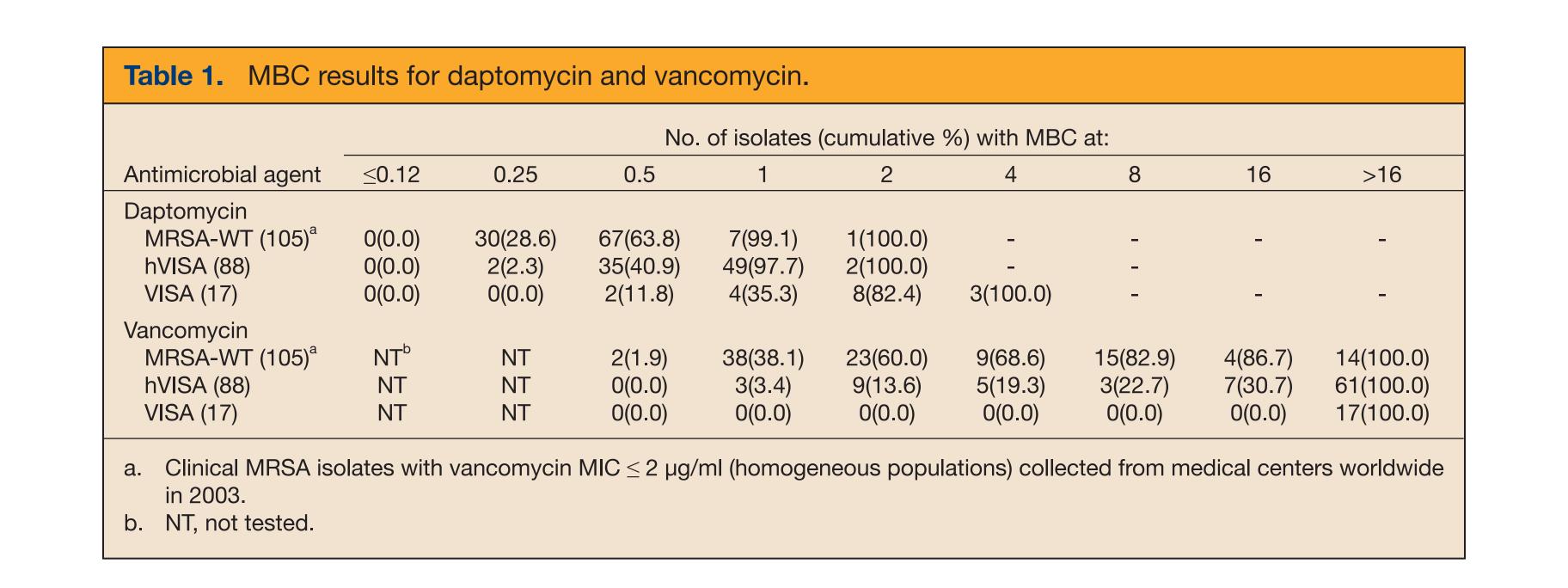
<u>Time Kill Curve</u>: Bactericidal activities of daptomycin and vancomycin were evaluated by time kill curve experiments on 15 selected strains and six controls according to Moody & Knapp [2004] and the NCCLS documents M21-A and M26-A. The selected strains included MRSA-WT with high vancomycin MBC/MIC ratios while the controls included MRSA-WT strains with vancomycin MBC/MIC ratios of 1 and ATCC strains. Bactericidal activity was defined as $\geq 3 \log_{10}$ reduction in the initial inoculum within 24 hours of incubation.

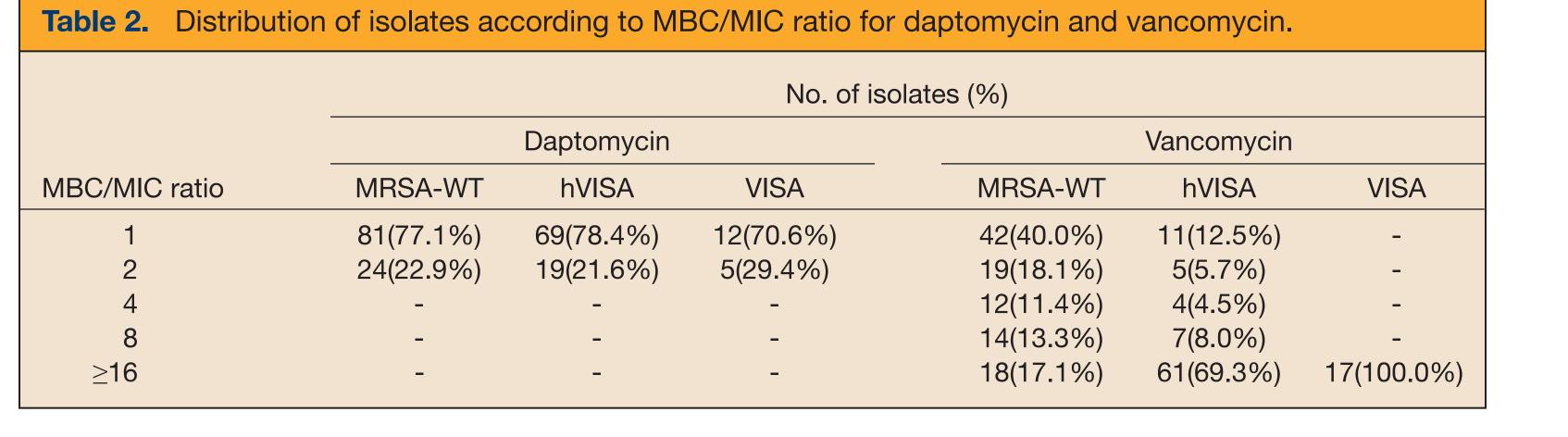
A. Vancomycin was tested at 2X, 4X, and 8X MIC with colony counts performed at T₀, T₄, T₈, T₂₄, and T₄₈. Six strains had the experiments repeated at 2X, 4X, 8X, 16X and 32X MIC with colony counts at T₀, T₄, T₈, and T₂₄.

B. Daptomycin was tested at 2X, 4X and 8X MIC with colony counts at T₀, T₄, T₈, and T₂₄.

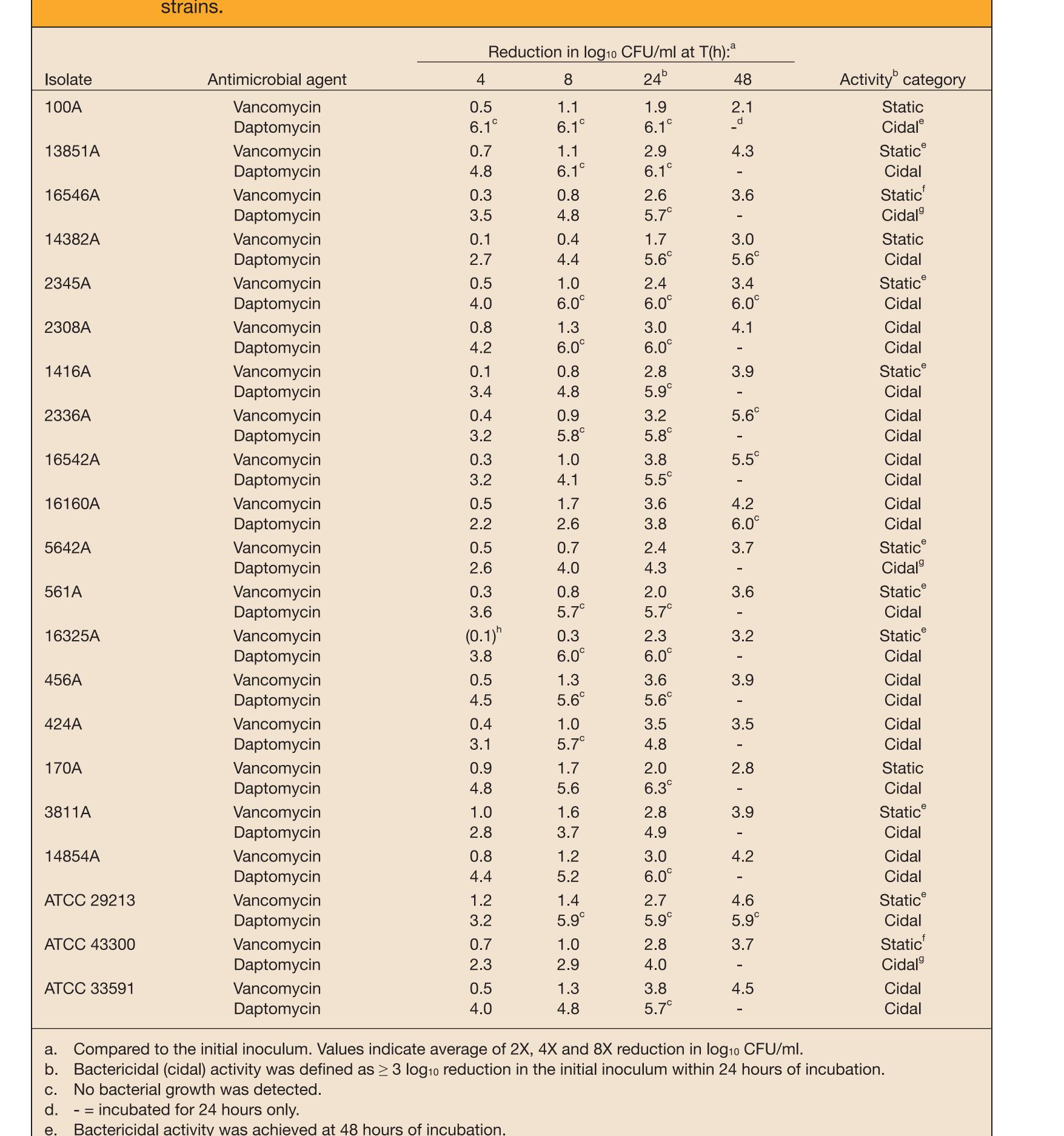
RESULTS

- The scattergram showing the results of the correlation between broth microdilution MIC and disk diffusion results for daptomycin is shown in Figure 1. Eight strains would be considered non-susceptible to daptomycin by the broth microdilution method and were susceptible by disk diffusion according to the current CLSI [2005] susceptible breakpoint (≤ 1 µg/ml and ≥ 16 mm). All eight strains were among the hVISA/VISA group. The correlation coefficient (r) was only 0.07.
- The scattergrams showing the results of the correlation between broth microdilution and disk diffusion results for vancomycin are shown in Figure 2. All isolates had inhibition zone diameters ≥ 16 mm and were considered susceptible according to the current CLSI [2005] susceptible breakpoint for vancomycin (≥ 15 mm). The inhibition zone diameter varied from 16 to 19 mm among the seven VISA strains with broth microdilution MIC of 8 µg/ml. The correlation coefficient (r) was also very low (0.11).
- The Etest showed a slight tendency towards lower daptomycin MIC results when compared to the broth microdilution method (data not shown). The rates of major and very major error for the Etest method was 1.9% when testing hVISA/VISA against daptomycin. No error was detected when testing MRSA-WT strains.
- All selected WT-MRSA strains showed an increase in the vancomycin and teicoplanin MIC results when tested by Etest with high inoculum in BHI agar (data not shown). However; according to the criteria proposed by Wootton et al. (2001), only one strain was considered hVISA (strain 16546A, MIC values ≥ 8 μg/ml for both vancomycin and teicoplanin after 48 hours of incubation).
- All MRSA-WT and hVISA strains were inhibited by \leq 1 µg/ml of daptomycin. However, a slight skewing toward a higher daptomycin MIC result was noted when the hVISA (MIC₅₀, 0.5 µg/ml and MIC₉₀, 1 µg/ml) and VISA (MIC₅₀, 1 µg/ml and MIC₉₀, 2 µg/ml) strains were compared to the MRSA-WT group (MIC₅₀ and MIC₉₀ of 0.5 µg/ml). Among the 17 VISA strains evaluated, seven strains (41.2%) showed a daptomycin MIC of 2 µg/ml and one (5.9%) strain had a daptomycin MIC of 4 µg/ml.
- 93.3% of isolates showed a daptomycin MBC of \leq 1 µg/ml with the highest daptomycin MBC result observed at 4 µg/ml (three isolates; Table 1). Eight of 11 daptomycin MBC results of 2 µg/ml and all three MBC results of 4 µg/ml were observed among the VISA strains.
- Only 60.0% of the MRSA-WT isolates showed vancomycin MBC results of \leq 2 µg/ml, the proposed CLSI (2006) vancomycin-susceptible breakpoint (Table 1). Furthermore, only 13.6% of the hVISA and none of the VISA strains showed MBC results at \leq 2 µg/ml. The number of occurrences (percentage) of isolates with vancomycin MBC result at \geq 16 µg/ml (proposed CLSI resistant breakpoint [CLSI, 2006]) were 18 (17.1%), 68 (77.3%), and 17 (100.0%) among the MRSA-WT, hVISA, and VISA groups, respectively (Table 1).
- All daptomycin MBC results were at the MIC or two-fold higher than the MIC, and the MBC/MIC ratios were not significantly affected by the susceptibility to vancomycin (Table 2). All three groups (MRSA-WT, hVISA and VISA) showed very similar MBC/MIC ratio results for daptomycin. Conversely, 17.1% of MRSA-WT strains, 69.3% of hVISA and all VISA strains showed a vancomycin MBC/MIC ratio of ≥ 16, e.g. tolerance.
- Vancomycin exhibited only modest and slow killing (Table 3 and Figure 3a). Reduction of CFU/ml was generally around one log₁₀ after 8 hours of incubation and around three log₁₀ after 24 hours of incubation. Among the 15 MRSA-WT strains that showed vancomycin tolerance in the previous study, vancomycin showed only bacteriostatic effect at all three concentrations (2X, 4X, and 8X MIC) in seven of them (47%).
- Daptomycin generally showed potent and rapid bactericidal activity (Table 3 and Figure 3b). At 4X MIC and 8X MIC, bactericidal activity was achieved after 4 hours of incubation for 15 (71%) and 19 (90%) strains, respectively. After 8 hours of incubation, ≥ 3 log₁₀ reduction in the initial inoculum was observed on 20 strains (95%) at 8X MIC, 19 strains at 4X MIC (90%), and 17 strains (81%) at 2X MIC.





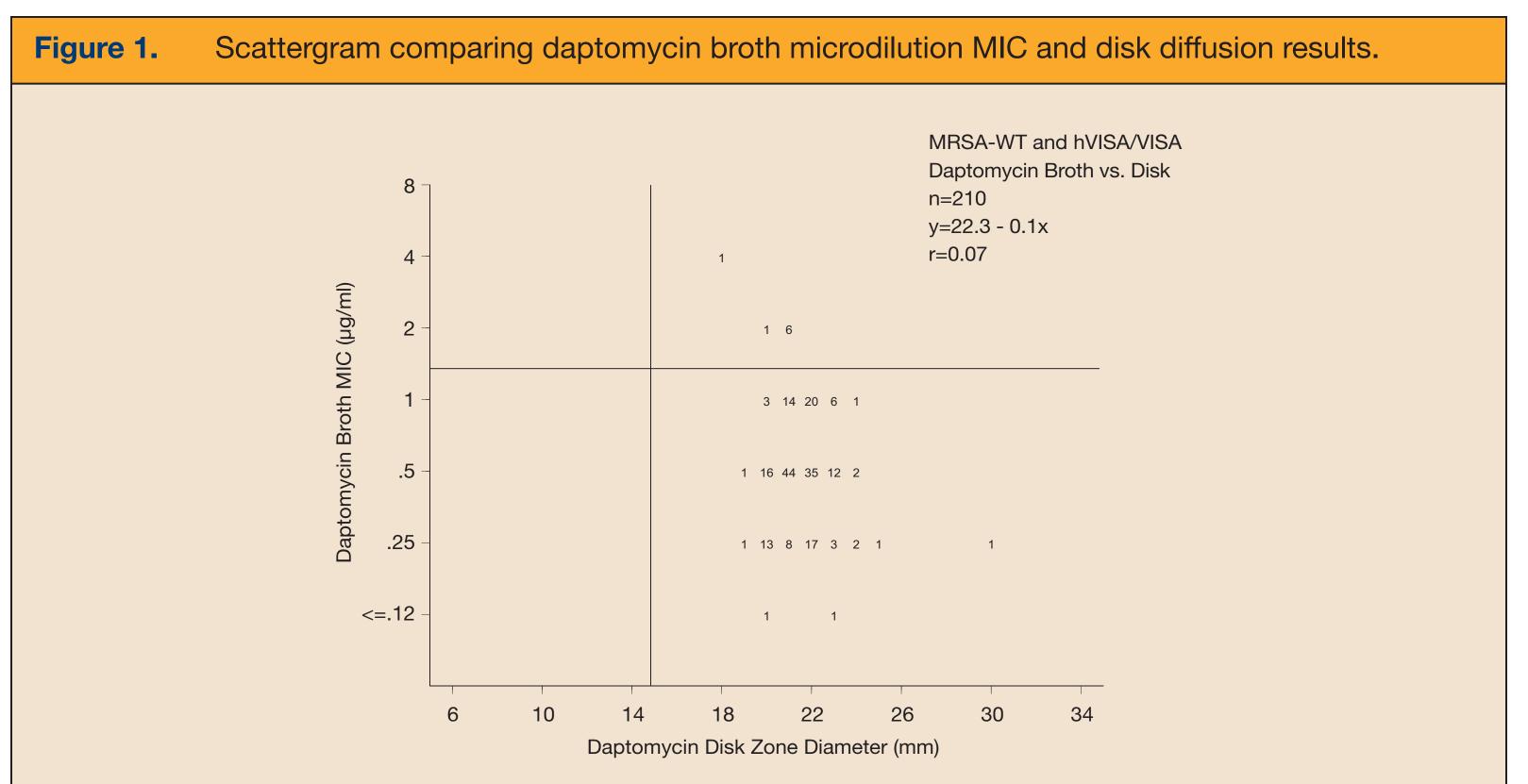
Kill curve results for vancomycin and daptomycin tested against oxacillin-resistant S. aureus

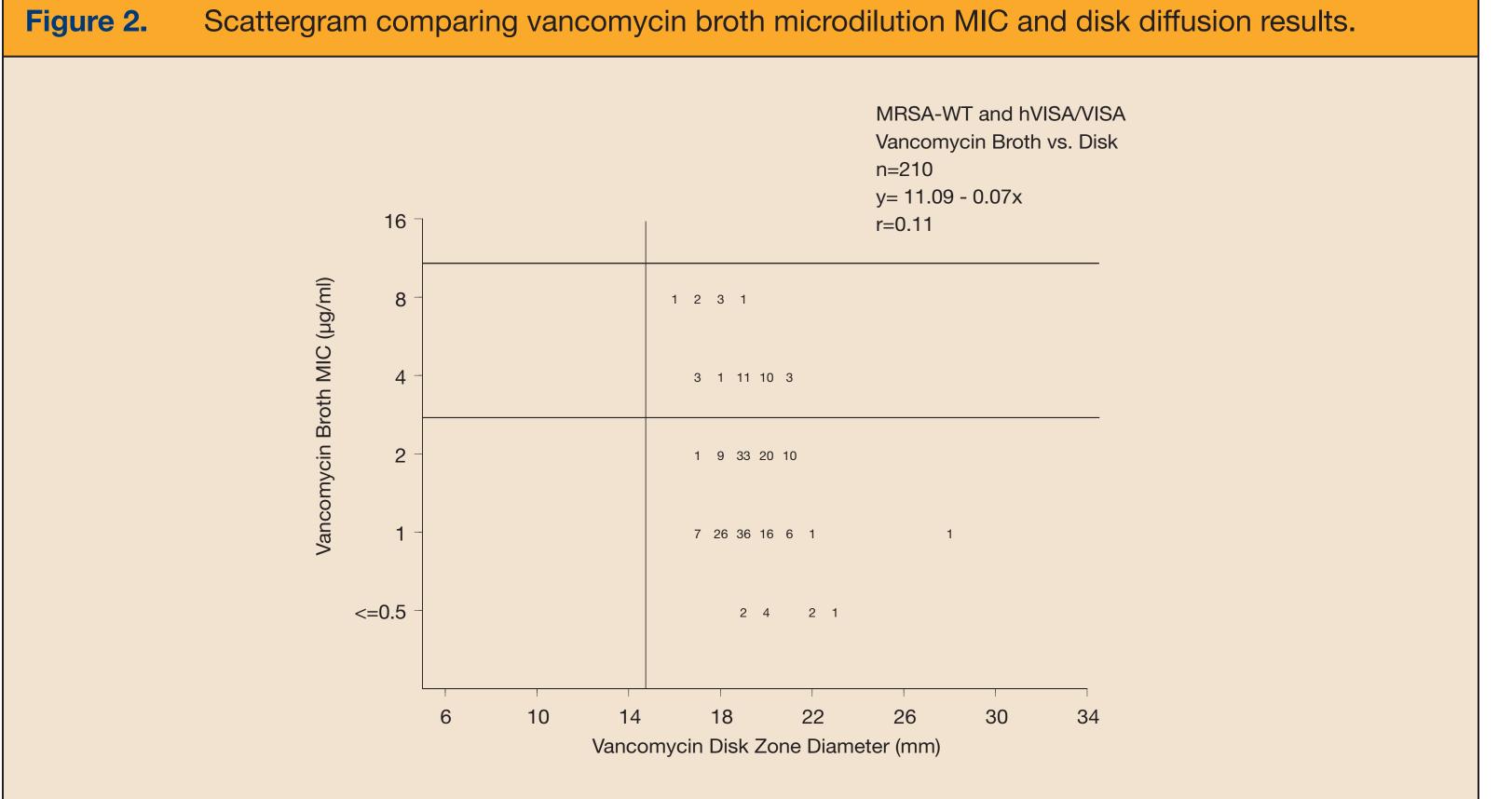


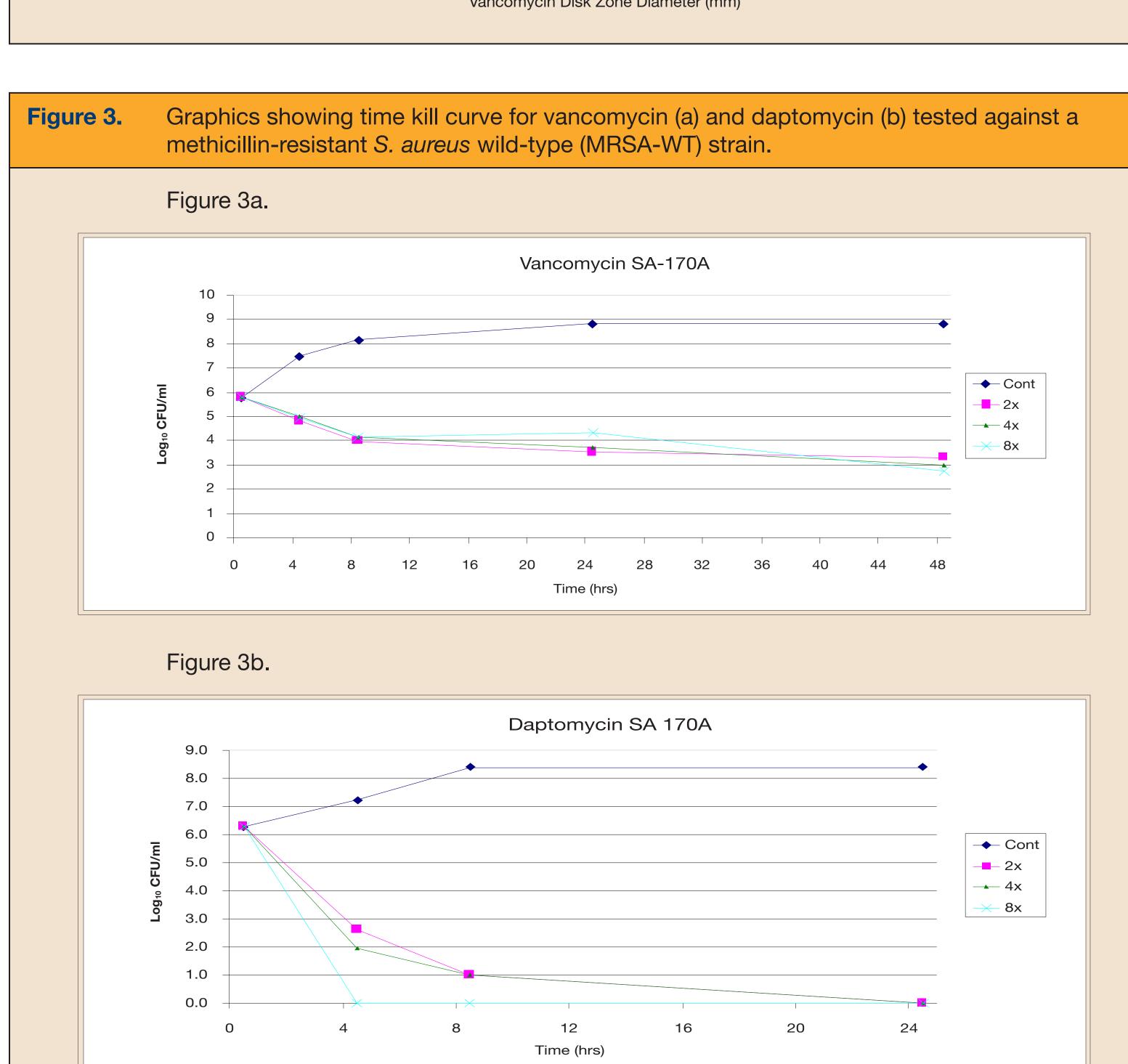
Bactericidal activity was achieved at 24 hours when tested at 4X and/or 8X MIC.

. Bacteriostatic activity when tested at 2X MIC.

n. Values in parenthesis represent increase in the log₁₀ CFU/ml.







CONCLUSIONS

- Daptomycin was highly bactericidal against *S. aureus* strains and its bactericidal activity was not affected by decreased susceptibility to vancomycin. In contrast, the majority of hVISA (69.3%) and all VISA strains showed vancomycin MBC/MIC ratios consistent with tolerance.
- A slight trend towards high daptomycin MIC results was observed among the hVISA/VISA strains, mainly among the VISA subset.
- The disk diffusion method generally failed to detect strains with elevated MIC values for vancomycin (\geq 8 µg/ml) or daptomycin (\geq 2 µg/ml).
- Elevated vancomycin MBC/MIC ratio could not be attributed to the presence of subpopulations with elevated vancomycin MIC (hVISA) and may represent a characteristic present in a high proportion of wild-type MRSA strains.
- Kill curve studies demonstrated that vancomycin has only a modest and slow killing activity against MRSA strains.
- Daptomycin showed rapid and potent bactericidal activity against MRSA strains when evaluated by time kill curve experiments. Increased vancomycin MBC/MIC ratio did not affect daptomycin bactericidal activity.

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