Evaluation of Bactericidal Activity of Daptomycin and Vancomycin Tested Against *Staphylococcus aureus* (VISA, hVISA and wild-types)

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ABSTRACT

Background: Daptomycin, a cyclic lipopeptide, was recently released for clinical treatment of serious Gram-positive infections.

Methods: 105 *S. aureus* with decreased susceptibility to vancomycin (88 heterogeneous [h] vancomycin-intermediate *S. aureus* [VISA] and 17 VISA) and 105 wild-type methicillin-resistant *S. aureus* (WT-MRSA) with vancomycin MIC ≤ 2 µg/ml were susceptibility tested by reference methods against daptomycin and vancomycin. The lowest concentration of antimicrobial that killed >99.9% of the initial inoculum was defined as the MBC. Tolerance was defined as a MBC/MIC ratio >16 and a resistant vancomycin MBC (≥ 32 µg/ml).

Results: All MRSA-WT and hVISA strains were inhibited by ≤ 1 µg/ml of daptomycin, while the VISA strains showed slightly higher daptomycin MICs (range, 0.5 – 4 µg/ml). The highest daptomycin MBC observed was only 4 µg/ml (3 isolates) and 93.3% of isolates showed daptomycin MBC ≤ 1 µg/ml. Among the MRSA-WT, hVISA and VISA groups, only 68.6, 19.3 and 5.8% respectively showed vancomycin MBC results ≤ 4 µg/ml. Fourteen (13.3%), 61 (69.3%), and 16 (94.2%) strains showed vancomycin MBC result ≥ 32 µg/ml among the MRSA-WT, hVISA, and VISA groups, respectively. Daptomycin MBC/MIC ratios were not significantly affected by vancomycin susceptibility. All daptomycin MBC results were at or only 2-fold greater than the MIC. Conversely, 17.1% of contemporary WT-MRSA strains, 69.3% of hVISA and all of VISA strains showed a vancomycin MBC/MIC ratio consistent with tolerance.

Conclusions: Daptomycin was highly bactericidal against *S. aureus*, including VISA and hVISA strains. Vancomycin showed only bacteriostatic activity against the vast majority of VISA and hVISA, and 17% of WT-MRSA exhibited tolerance.

INTRODUCTION

Bacteria within cardiac vegetations may reach very high concentrations (10^8 - 10^{10} organisms per gram of tissue). At such densities, rates of metabolism and cell division appear to be reduced, resulting in a reduced susceptibility to bactericidal effects of cell wall-active agents. The bacteria are dormant, being surrounded by fibrin, platelets, and possibly calcified material. Bacteria considered susceptible to various antimicrobials in most situations are relatively resistant in endocarditis. Clinical cure is often achieved, but prolonged administration of relatively high doses of a bactericidal cell wall-active antibacterial agent is generally required for true sterilization of the vegetation to kill any dormant bacteria when they start to produce cell walls with division.

Vancomycin, the first glycopeptide antimicrobial agent, has been studied for five decades and susceptibility testing results document continued in vitro activity against a wide variety of Gram-positive cocci. However, more recently the emergence of vancomycin-intermediate *S. aureus* (VISA) and hetero-VISA strains has questioned the efficacy of this antimicrobial in the treatment of staphylococcal infections. Also, there have been several studies demonstrating that vancomycin bactericidal activity is significantly reduced during bacterial stationary phase, under anaerobic conditions, and at an increased inoculum.

Daptomycin has rapid in vitro bactericidal activity against a wide spectrum of Gram-positive organisms, including multidrug-resistant (MDR) strains of staphylococci, streptococci and enterococci. Daptomycin has a unique mechanism of action with no cross resistance to glycopeptide (teicoplanin and vancomycin) resistant strains. This compound has been recently approved by the United States Food and Drug Administration (FDA) for treatment of complicated skin and soft tissue 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.

We evaluated the bactericidal activities of daptomycin and vancomycin against vancomycin-intermediate (VISA) and hetero-VISA *S. aureus* strains as compared to a MRSA collection of wild type (WT) strains.

MATERIALS AND METHODS

Organism collection: A collection of 210 strains was selected for the study. The collection is composed of two groups of strains:

A. MRSA-WT group: 105 oxacillin-resistant *S. aureus* strains 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.

B. hVISA/VISA group: 105 isolates

1. hVISA subset: Includes 88 isolates with vancomycin MIC results ≤ 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; www.narsa.net)

Susceptibility testing: MIC values were determined by broth microdilution methods for daptomycin, vancomycin and oxacillin with appropriate medium variations (50 mg/L of calcium) for testing daptomycin. MBC values were assessed for daptomycin and vancomycin by plating all (0.1 ml) of the broth from the clear MIC well and from the log_2 dilutions greater than 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 kills ≥ 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 ≥ 32 µg/ml for vancomycin (resistant). Quality control strains (*S. aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) were tested along with every set of tests.

RESULTS

- All MRSA-WT and hVISA strains were inhibited by ≤ 1 µg/ml of daptomycin (Figure 1). Nevertheless, a slight skewing toward a higher daptomycin MIC result was noted when the hVISA (MIC_{50}, 0.5 µg/ml and MIC_{90}, 1 µg/ml) and VISA (MIC_{50}, 1 µg/ml and MIC_{90}, 2 µg/ml) strains were compared to the MRSA-WT group (MIC_{50} and MIC_{90} of 0.5 µg/ml).
- The highest daptomycin MBC result observed was 4 µg/ml (three isolates) and 93.3% of isolates showed a daptomycin MBC of ≤ 1 µg/ml (Figure 2 and 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 68.6% of the MRSA-WT isolates showed vancomycin MBC results of ≤ 4 µg/ml, the current CLSI/NCCLS vancomycin-susceptible breakpoint (Figure 2). Furthermore, only 19.3% of the hVISA and none of the VISA strains showed vancomycin MBC results at ≤ 4 µg/ml.
- The number of occurrences (percentage) of isolates with vancomycin MBC results at ≥ 32 µg/ml (CLSI/NCCLS resistant breakpoint) were 14 (13.3%), 61 (69.3%), and 17 (100.0%) among the MRSA-WT, hVISA, and VISA groups, respectively (Figure 2).

Table 1. MIC and MBC results for three subsets of *S. aureus* strains.

	No. of isolates (%)					
	MRSA-WT (105)		hVISA (88)		VISA (17)	
Daptomycin concentration (µg/ml)[a]	MIC	MBC	MIC	MBC	MIC	MBC
≤0.12	2 (2)	-	-	-	-	-
0.25	44 (42)	30 (29)	2 (2)	2 (2)	-	-
0.5	56 (53)	67 (64)	52 (59)	35 (40)	2 (12)	2 (12)
Susceptible 1	3 (3)	7 (7)	34 (39)	49 (56)	7 (41)	4 (24)
2	-	1 (1)	-	2 (2)	7 (41)	8 (47)
4	-	-	-	-	1 (6)	3 (18)
8	-	-	-	-	-	-
Vancomycin concentration (µg/ml)[a]	MIC	MBC	MIC	MBC	MIC	MBC
≤0.5	9 (9)	2 (2)	-	-	-	-
1	84 (80)	38 (36)	9 (10)	3 (3)	-	-
2	12 (11)	23 (22)	61 (69)	9 (10)	-	-
Susceptible 4	-	9 (9)	18 (21)	5 (6)	11 (65)	-
8	-	15 (14)	-	3 (3)	6 (35)	-
16	-	4 (4)	-	7 (8)	-	-
Resistant ≥32	-	14 (13)	-	61 (69)	-	17 (100)

a. Broken lines indicate breakpoints for staphylococci (CLSI, 2005).

- All daptomycin MBC results were at the MIC or two-fold higher than the MIC, and the MBC/MIC ratio were not significantly affected by the susceptibility to vancomycin (Figure 3). 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 tolerance to vancomycin.

Figure 1a. MIC results for daptomycin and vancomycin when tested against MRSA-wild type strains.

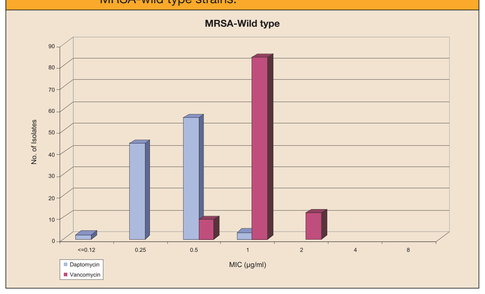


Figure 2a. MBC results for daptomycin and vancomycin when tested against MRSA-wild type strains.

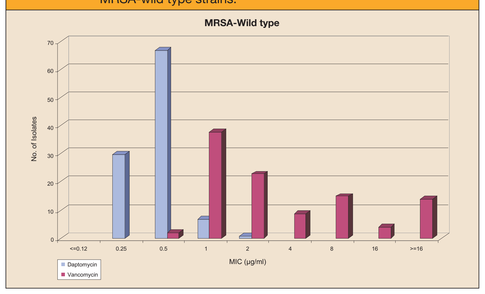


Figure 1b. MIC results for daptomycin and vancomycin when tested against hetero-VISA strains.

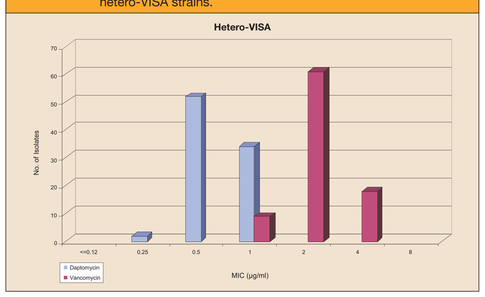


Figure 2b. MBC results for daptomycin and vancomycin when tested against hetero-VISA strains.

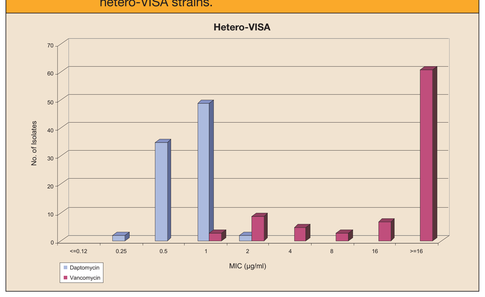


Figure 3a. Distribution of MRSA-wild type isolates according to MBC/MIC ratio for daptomycin and vancomycin.

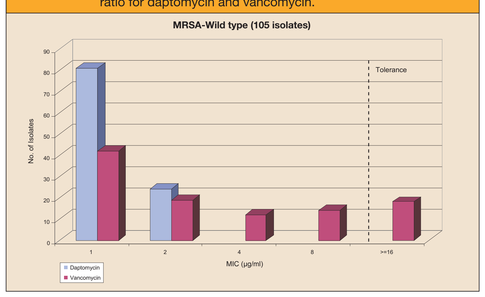


Figure 1c. MIC results for daptomycin and vancomycin when tested against VISA strains.

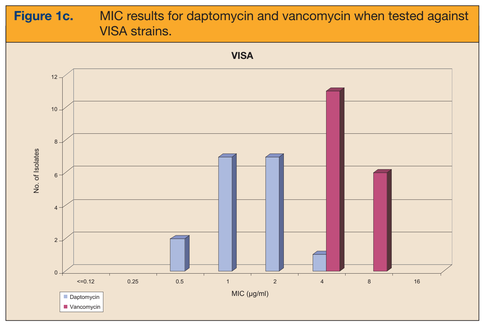


Figure 2c. MBC results for daptomycin and vancomycin when tested against VISA strains.

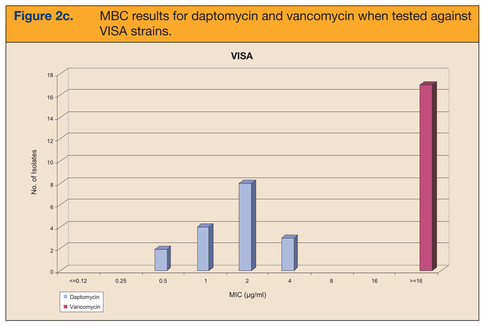
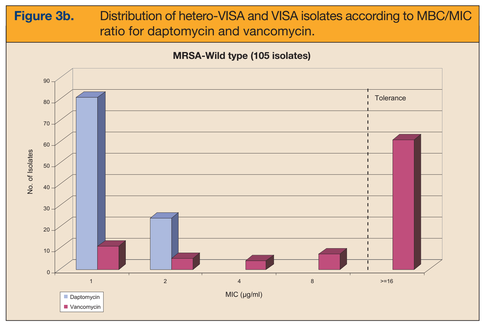


Figure 3b. Distribution of hetero-VISA and VISA isolates according to MBC/MIC ratio for daptomycin and vancomycin.



CONCLUSIONS

- Daptomycin was bactericidal against all *S. aureus* strains and its bactericidal activity was not significantly influenced by decreased susceptibility to vancomycin.
- A slight trend towards high daptomycin MIC results was observed among the hVISA/VISA strains, mainly among the VISA subset.
- A high vancomycin MBC/MIC ratio, consistent with tolerance, was observed in an elevated proportion (17.1%) of wild-type (non-VISA, non-hVISA) *S. aureus* strains.
- The clear majority of hVISA (69.3%) and all VISA strains demonstrated vancomycin MBC/MIC ratios consistent with drug tolerance.
- The efficacy of vancomycin in the treatment of *S. aureus* endocarditis may be compromised by its decreased bactericidal activity (high MBC/MIC values).

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