Bactericidal Activity of Vancomycin and Daptomycin Tested Against Heterogeneous and Homogeneous Vancomycin-Intermediate *S. aureus* (hVISA and VISA) Strains

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ABSTRACT

Objective: To evaluate vancomycin (VAN) and daptomycin (DAP) bactericidal activity tested against *S. aureus* strains with decreased susceptibility to VAN (VISA and hVISA).

Methods: A total of 105 well-characterized *S. aureus* strains with decreased susceptibility to VAN (88 hVISA and 17 VISA) as well as 105 oxacillin-resistant wild-type (WT) *S. aureus* with VAN MIC ≤ 2 mg/L (MRSA-WT; isolated in 2003) were susceptibility (S) tested by reference NCCLS broth microdilution method against DAP and VAN. MBC values were assessed by plating the entire volume of the broth from each well above the MIC for each organism. The lowest concentration of antimicrobial agent that killed ≥ 99.9% of the initial inoculum was defined as the MBC endpoint. Bactericidal action was defined as a MBC/MIC ratio ≤ 2 and tolerance was defined as a MBC/MIC ratio ≥ 16 and a resistant (R) VAN MBC (≥ 32 mg/L).

Results: VAN MIC and MBC results for 3 subsets of *S. aureus* strains are summarized in the Table.

	No. of isolates (cumulative %)					
	VISA (17)		hVISA (88)		MRSA-WT (105)	
VAN concentration (mg/L)	MIC	MBC	MIC	MBC	MIC	MBC
≤0.5	-	-	-	-	9 (8.6)	2 (1.9)
1	-	-	9 (10.2)	3 (3.4)	84 (88.6)	38 (38.1)
2	-	-	61 (79.5)	9 (14.9)	12 (100.0)	23 (60.0)
Susceptible 4	11 (64.7)	-	18 (100.0)	5 (19.3)	-	9 (68.6)
8	6 (33.3)	-	-	3 (22.7)	-	15 (82.9)
16	-	-	-	7 (30.7)	-	4 (86.7)
Resistant ≥32	-	17 (100.0)	-	61 (100.0)	-	14 (100.0)

Only two-thirds of the MRSA-WT isolates showed VAN MBC results ≤ 4 mg/L, and for hVISA and VISA groups, only 19.3 and 0.0% of isolates had a VAN MBC at ≤ 4 mg/L, respectively. Fourteen (13.3%), 61 (69.3%), and 17 (100.0%) strains respectively showed VAN MBC result ≥ 32 mg/L among the MRSA-WT, hVISA, and VISA groups. In contrast, all MRSA-WT and hVISA strains were inhibited by ≤ 1 mg/L of DAP, while the VISA strains showed slightly higher DAP MIC values (range, 0.5 – 4 mg/L). The highest DAP MBC result was only 4 mg/L and 93.3% of isolates showed a DAP MBC at ≤ 1 mg/L. Eight of 11 DAP MBC results of 2 mg/L and all 3 MBC results of 4 mg/L were observed among the VISA strains. DAP MBC/MIC ratio was not significantly affected by VAN susceptibility. All DAP MBC results were at the MIC or only two-fold greater. 17.1% of MRSA-WT strains, 69.3% of hVISA and all VISA strains showed a VAN MBC/MIC ratio consistent with tolerance.

Conclusions: VAN showed bacteriostatic activity against the majority of VISA and hVISA strains. VAN also had high rates of tolerant MBC/MIC ratios, but DAP was highly bactericidal against MRSA-WT as well as VISA and hVISA strains.

INTRODUCTION

Daptomycin is a cyclic lipopeptide approved by the US-FDA for the treatment of complicated skin and skin structure infections caused by oxacillin-susceptible and -resistant *S. aureus* and ß-haemolytic streptococci with a daptomycin MIC breakpoint at ≤ 1 mg/L, and vancomycin-susceptible *E. faecalis* with a breakpoint at ≤ 4 mg/L. Daptomycin is also active against a wide range of multidrug-resistant (MDR) strains such as vancomycin-resistant enterococci and methicillin-resistant *S. aureus,* for which there are few therapeutic alternatives.

Daptomycin has a unique mechanism of action with no cross resistance to other antimicrobials. Daptomycin acts on the cytoplasmic membrane in the presence of physiological levels of calcium ions and therefore in vitro susceptibility testing requires appropriate supplementation of calcium to the test media (50 mg/L in broth). The Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) recommends mueller-hinton broth with 50 mg/L of calcium.

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) clinical isolates.

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. hVISA/VISA group: 105 isolates
   1. hVISA subset: Includes 88 isolates with vancomycin MIC results ≤ 4 mg/L by reference broth microdilution method that show a subpopulation with a vancomycin MIC result > 4 mg/L when tested using a high inoculum (heterogeneous population).
   2. VISA subset: Includes 17 isolates with vancomycin MIC results of 4 or 8 mg/L. 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)
B. MRSA-WT group: 105 oxacillin-resistant *S. aureus* strains with vancomycin MIC results ≤ 2 mg/L (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.

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 mg/L 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 mg/L of daptomycin. However, a slight skewing toward a higher daptomycin MIC result was noted when the hVISA (MIC_{50}, 0.5 mg/L and MIC_{90}, 1 mg/L) and VISA (MIC_{50}, 1 mg/L and MIC_{90}, 2 mg/L) strains were compared to the MRSA-WT group (MIC_{50} and MIC_{90} of 0.5 mg/L). Among the 17 VISA strains evaluated, seven strains (41.2%) showed a daptomycin MIC of 2 mg/L and one (5.9%) strain had a daptomycin MIC of 4 mg/L (Table 1).

Table 1. MIC results for daptomycin and vancomycin.

	No. of isolates (cumulative %) inhibited at:						
Antimicrobial agent	≤0.12	0.25	0.5	1	2	4	8
Daptomycin							
MRSA-WT (105)[a]	2(1.9)	44(43.8)	56[b](97.1)	3(100.0)	-	-	-
hVISA (88)	0(0.0)	2(2.3)	52(61.4)	34(100.0)	-	-	-
VISA (17)	0(0.0)	0(0.0)	2(11.8)	7(52.9)	7(94.1)	1(100.0)	-
Vancomycin							
MRSA-WT (105)[a]	NT[c]	NT	9(8.6)	84(88.6)	12(100.0)	-	-
hVISA (88)	NT	NT	0(0.0)	9(10.2)	61(79.5)	18(100.0)	-
VISA (17)	NT	NT	0(0.0)	0(0.0)	0(0.0)	11(64.7)	6(100.0)

a. Clinical MRSA isolates with vancomycin MIC ≤ 2 mg/L collected from medical centers worldwide in 2003.
b. The underline indicates the modal value.
c. NT, not tested.

Table 2. MBC results for daptomycin and vancomycin.

	No. of isolates (cumulative %) with MBC at:								
Antimicrobial agent	≤0.12	0.25	0.5	1	2	4	8	16	≥32
Daptomycin									
MRSA-WT (105)[a]	0(0.0)	30(28.6)	67(63.8)	7(99.1)	1(100.0)	-	-	-	-
hVISA (88)	0(0.0)	2(2.3)	35(40.9)	49(97.7)	2(100.0)	-	-	-	-
VISA (17)	0(0.0)	0(0.0)	2(11.8)	4(35.3)	8(82.4)	3(100.0)	-	-	-
Vancomycin									
MRSA-WT (105)[a]	NT[b]	NT	2(1.9)	38(38.1)	23(60.0)	9(68.6)	15(82.9)	4(86.7)	14(100.0)
hVISA (88)	NT	NT	0(0.0)	3(3.4)	9(14.9)	5(19.3)	3(22.7)	7(30.7)	61(100.0)
VISA (17)	NT	NT	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	17(100.0)

a. Clinical MRSA isolates with vancomycin MIC ≤ 2 mg/L collected from medical centers worldwide in 2003.
b. NT, not tested.

- The highest daptomycin MBC result observed was 4 mg/L (three isolates) and 93.3% of isolates showed a daptomycin MBC of ≤ 1 mg/L (Tables 2 and 3). Eight of 11 daptomycin MBC results of 2 mg/L and all three MBC results of 4 mg/L were observed among the VISA strains.

- Only 68.6% of the MRSA-WT isolates showed vancomycin MBC results of ≤ 4 mg/L, the current CLSI/NCCLS vancomycin-susceptible breakpoint (Table 2). Furthermore, only 19.3% of the hVISA and none of the VISA strains showed vancomycin MBC results at ≤ 4 mg/L. The number of occurrences (percentage) of isolates with vancomycin MBC result at ≥ 32 mg/L (CLSI/NCCLS resistant breakpoint) were 14 (13.3%), 61 (69.3%), and 17 (100.0%) among the MRSA-WT, hVISA, and VISA groups, respectively (Table 2).

- 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 (Table 4). 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 consistent with tolerance.

Table 3. Daptomycin MIC and MBC results for three subsets of *S. aureus* strains.

	MRSA-WT (105)		hVISA (88)		VISA (17)	
Daptomycin concentration (mg/L)[a]	MIC	MBC	MIC	MBC	MIC	MBC
≤0.12	2	-	-	-	-	-
0.25	44	30	2	2	-	-
0.5	56	67	52	35	2	2
Susceptible 1	3	7	34	49	7	4
2	-	1	-	2	7	8
4	-	-	-	-	1	3
8	-	-	-	-	-	-

a. Broken line indicates daptomycin susceptible breakpoint for staphylococci. (CLSI, 2005).

Table 4. Distribution of isolates according to MBC/MIC ratio for daptomycin and vancomycin.

	No. of isolates (%)					
	Daptomycin			Vancomycin		
MBC/MIC ratio	MRSA-WT	hVISA	VISA	MRSA-WT	hVISA	VISA
1	81(77.1)	69(78.4)	12(70.6)	42(40.0)	11(12.5)	-
2	24(22.9)	19(21.6)	5(29.4)	19(18.1)	5(5.7)	-
4	-	-	-	12(11.4)	4(4.5)	-
8	-	-	-	14(13.3)	7(8.0)	-
≥16	-	-	-	18(17.1)	61(69.3)	17(100.0)

CONCLUSIONS

- A slight trend towards higher daptomycin MIC results was observed among the hVISA/VISA strains, mainly among the VISA subset, compared to the MRSA-WT strains.
- Daptomycin was bactericidal against *S. aureus* strains and its bactericidal activity was not significantly influenced by decreased susceptibility to vancomycin.
- 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.

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