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The Evaluation of the Bactericidal Activity of Daptomycin, Vancomycin and Linezolid and Determination of the Interactions of These Antimicrobials with Gentamicin or Rifampin against *S. aureus*

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ABSTRACT

Objective: To determine the bactericidal activities of daptomycin (DAP), vancomycin (VAN) and linezolid (LZD), and the drug interaction categories for these 3 antimicrobials combined with each other or tested with gentamicin (GEN) or rifampin (RIF) against *S. aureus* strains.
Methods: Minimum inhibitory concentrations (MIC) were determined by CLSI broth microdilution methods. Bactericidal activity (MBC) was evaluated on 207 *S. aureus* (101 wild-type [WT] oxacillin-resistant [MRSA], 64 VAN-heteroresistant [hVISA], 37 VAN-intermediate [VISA] and 5 VAN-resistant [VRSA]) by plating the entire broth content from the well onto appropriate growth media. Quantitative colony counts were performed and compared to the initial inoculum. Checkerboard synergy tests were performed on 18 *S. aureus* (15 MRSA) using broth microdilution trays containing 2 agents in 2-fold dilutions dispensed in a checkerboard format. DAP, VAN and LZD were combined with each other and with GEN or RIF, resulting in 9 combinations. The fractional inhibitory concentration (FIC) was calculated for each agent and the summation of both FICs was used to classify the combined activity of antimicrobials as synergistic (SYN; ≤0.5), partially synergistic (PSYN; >0.5 and <1), additive (ADD; 1), indifferent (IND; >1 and <4) and antagonistic (ANT; ≥4).
Results: DAP was bactericidal against all *S. aureus* groups while VAN MBC/MIC ratios consistent with tolerance were observed at a rate of 14.9; 68.8; and 86.5% for WT-MRSA, hVISA and VISA, respectively, and LZD MBC/MIC ratios consistent with tolerance were observed at a rate of 99.0; 100.0; 94.6 and 100.0% for WT-MRSA, hVISA, VISA and VRSA, respectively. Checkerboard results showed no ANT or SYN with any of the antimicrobial combinations evaluated. The majority of strains (77.8%) showed PSYN (50.0%) or ADD (28.8%) interactions when DAP was combined with GEN; while ADD and IND effects predominated when DAP was combined with LZD (94.4%), RIF (88.9%) or VAN (72.2%). All isolates showed IND effect when LZD was combined with GEN. VAN combinations exhibited predominant ADD (16.7-38.9%) or IND interactions (44.4-77.8%).

Table. MBC results for daptomycin, linezolid and vancomycin tested against *S. aureus*.

	No. of isolates (cumulative %) with MBC at:								
Antimicrobial agent	≤0.12	0.25	0.5	1	2	4	8	16	≥32
Daptomycin									
MRSA-WT (101)[a]	0(0)	29(29)	64(92)	7(99)	1(100)	-	-	-	-
hVISA (64)	0(0)	1(2)	32(52)	30(98)	1(100)	-	-	-	-
VISA (37)	0(0)	0(0)	2(5)	23(65)	9(92)	3(100)	-	-	-
VRSA (5)	0(0)	0(0)	4(80)	1(100)	-	-	-	-	-
Linezolid									
MRSA-WT (101)[a]	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)	0(1)	100(100)
hVISA (64)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	64(100)
VISA (37)	0(0)	0(0)	0(0)	0(0)	2(5)	0(5)	0(5)	0(5)	35(100)
VRSA (5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	5(100)
Vancomycin[b]									
MRSA-WT (101)[a]	NT[c]	NT	2(2)	38(40)	23(62)	9(71)	14 (85)	3 (88)	12 (100)
hVISA (64)	NT	NT	0(0)	3(3)	9(12)	3(17)	2(20)	4(24)	43(100)
VISA (37)	NT	NT	0(0)	0(0)	0(0)	2(5)	1(8)	0(8)	34(100)

a. Clinical MRSA isolates with vancomycin MIC ≤2 mg/L (homogeneous populations) collected from medical centers worldwide in 2003.
b. MBC was not evaluated on VRSA strains (five strains with vancomycin MIC >32 mg/L).
c. NT, not tested.

Conclusions: DAP was the only agent highly bactericidal against *S. aureus* strains, independent of their susceptibility to VAN. The combinations of DAP with any other agent were never antagonistic. DAP with GEN and LZD with RIF generally exhibited a PSYN or ADD interaction; while IND and ADD interactions predominated among all other combinations.

INTRODUCTION

Infections caused by methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA) strains are associated with longer hospital stays, more days of antimicrobial therapy and higher costs than infections caused by methicillin-susceptible *S. aureus* (MSSA) strains. Vancomycin remains the standard for treating most MRSA infections; however, concerns over increases in the rates of heteroresistance and tolerance to this agent, combined with its safety and clinical shortcomings have motivated the development of newer agents.

Daptomycin is a novel lipopeptide antimicrobial agent which was approved by the United States Food and Drug Administration (US-FDA) and by the European Medicines Agency (EMEA) for the treatment of complicated skin and skin structure infections (cSSSI). More recently, daptomycin was approved for the treatment of *S. aureus* bacteremia and right-sided infectious endocarditis.

The activity of daptomycin has also been investigated in combination with other antimicrobial agents, most commonly tested against *Enterococcus* spp. Synergy has been observed against vancomycin-resistant enterococcal isolates treated with daptomycin plus rifampin. Furthermore, additive or synergistic effects have also been demonstrated with daptomycin plus ampicillin against *Enterococcus faecalis* and *E. faecium* isolates, as well as daptomycin plus gentamicin against an ampicillin-resistant *E. faecium* isolate. Most importantly, antagonism between daptomycin and other antimicrobial agents has not been reported.

The objective of this study was to determine the bactericidal activities of daptomycin, vancomycin and linezolid, and the drug interaction categories for these three antimicrobials when combined with each other or tested with gentamicin or rifampin against *S. aureus* strains.

MATERIALS AND METHODS

Bacterial strains: Bactericidal activity (MBC) was evaluated on 207 *S. aureus* strains that included 101 wild-type MRSA (MRSA-WT) strains with a homogenous population of vancomycin MIC results at ≤2 mg/L. These isolates were collected from more than 50 medical centers worldwide in 2003; no more than two strains per medical center were included. Secondly, a hVISA/VISA/VRSA group (106 isolates) was tested. The hVISA subset included 64 isolates with vancomycin MIC results ≤2 mg/L by reference broth microdilution method that show a subpopulation with vancomycin and teicoplanin MIC at ≥8 mg/L when tested with high inoculum (heterogeneous population). This collection has been well characterized by Wootton et al. (2001) and Howe et al. (2004). The VISA subset includes 37 isolates with vancomycin MIC results of 4 or 8 mg/L and a homogenous population; while the VRSA subset included five strains with vancomycin MIC >32 mg/L. Five of the VISA strains, as well as all five VRSA strains were provided by the Network on Antimicrobial Resistance in *S. aureus* (NARSA; www.narsa.net).

Checkerboard synergy testing was performed on 18 *S. aureus* strains, including 17 recent clinical isolates and *S. aureus* ATCC 29213.

Susceptibility testing: MIC values were determined by broth microdilution tests according to CLSI M7-A7 methods. Linezolid MIC values were read at the first well that showed a prominent reduction in growth. Quality control strains *S. aureus* ATCC 25923 *and E. faecalis* ATCC 29212 were tested concurrently with every set of tests.

MBC values were assessed by plating the entire broth content from the MIC well and from those wells four $\log_2$ dilutions above the MIC for each organism onto appropriate growth media. Quantitative colony counts were performed on the initial inoculum. The lowest concentration of antimicrobial agent that kills ≥99.9% of the starting test inoculum was defined as the MBC enpoint. Tolerance was defined as MBC/MIC ≥32 or MBC/MIC of 16 and MBC values ≥16 mg/L.

Synergy or drug interaction tests were performed in 96-well broth microdilution trays containing two antimicrobial agents in two-fold dilutions dispensed in a checkerboard format. The fractional inhibitory concentration (FIC) was calculated for each agent and the summation of both FICs was used to classify the combined activity of antimicrobials as synergistic (SYN; ≤0.5), partially synergistic (PSYN; >0.5 and <1), additive (ADD; 1), indifferent (IND; >1 and <4) and antagonistic (ANT; ≥4). The following nine combinations were evaluated: daptomycin and gentamicin; daptomycin and rifampin; vancomycin and gentamicin; vancomycin and rifampin; linezolid and gentamicin; linezolid and rifampin; daptomycin and vancomycin; linezolid and vancomycin; and daptomycin and linezolid.

RESULTS

- Daptomycin was highly active against MRSA-WT (MIC_{50} and MIC_{90} of 0.5 mg/L), hVISA (MIC_{50}, 0.5 mg/L and MIC_{90}, 1 mg/L) and VRSA (all MIC values at 0.5 mg/L) strains (Table 1). VISA strains showed daptomycin MIC values slightly higher (one $\log_2$ dilution) compared to other groups.
- Daptomycin showed potent bactericidal activity against MRSA-WT (MBC_{50} and MBC_{90} of 0.5 mg/L), hVISA (MBC_{50}, 0.5 mg/L and MBC_{90}, 1 mg/L) and VRSA (MBC_{50} and MBC_{90} of 1 mg/L) strains; while VISA strains showed daptomycin MBC values slightly higher (MBC_{50}, 1 mg/L and MBC_{90}, 2 mg/L).
- The vast majority of strains tested (98.6%) showed linezolid MBC/MIC values ≥32, which is consistent with the agent's bacteriostatic action (Table 2).
- Vancomycin MBC values were relatively high for all groups. Only 62.4% of MRSA-WT and 11.9% of hVISA strains showed vancomycin MBC values ≤2 mg/L, which is the revised vancomycin susceptible breakpoint established by the CLSI.
- All daptomycin MBC results were at the MIC or only two-fold higher than the MIC (except one MRSA-WT strain with a MBC/MIC of 4). Furthermore, the MBC/MIC ratio was not adversely affected by the susceptibility pattern of vancomycin (Table 2).
- A clear majority of strains (77.8%) showed partial synergy (50.0%) or additive (27.8%) interactions when daptomycin was combined with gentamicin; while additive and indifferent effects predominated when daptomycin was combined with linezolid (94.4%), rifampin (88.9%) or vancomycin (72.2%; seeTable 3).

Table 1. MIC results for daptomycin, vancomycin and linezolid tested against 207 *S. aureus* strains.

	No. of isolates (cumulative %) inhibited at:							
Antimicrobial agent	≤0.12	0.25	0.5	1	2	4	8	≥16
Daptomycin								
MRSA-WT (101)[a]	2(1.9)	41(42.6)	55[b](97.0)	3(100.0)	-	-	-	-
hVISA (64)	0(0.0)	1(1.6)	46(73.4)	17(100.0)	-	-	-	-
VISA (37)	0(0.0)	0(0.0)	5(13.6)	24(78.4)	7(97.3)	1(100.0)	-	-
VRSA (5)	0(0.0)	0(0.0)	5(100.0)	-	-	-	-	-
Linezolid								
MRSA-WT (101)[a]	0(0.0)	0(0.0)	0(0.0)	6(5.9)	94(99.0)	1(100.0)	-	-
hVISA (64)	0(0.0)	0(0.0)	0(0.0)	37(57.8)	27(100.0)	-	-	-
VISA (37)	0(0.0)	0(0.0)	0(0.0)	29(78.4)	8(100.0)	-	-	-
VRSA (5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(100.0)	-	-	-
Vancomycin								
MRSA-WT (101)[a]	NT[c]	NT	9(8.9)	81(89.1)	11(100.0)	-	-	-
hVISA (64)	-	-	-	8(12.5)	56(87.5)	-	-	-
VISA (37)	-	-	-	-	-	30(81.1)	7(18.9)	-
VRSA (5)	-	-	-	-	-	-	-	5(100.0)[d]

a. Clinical MRSA isolates with vancomycin MIC ≤2 mg/L (homogeneous populations) collected from medical centers worldwide in 2003.
b. The underline indicates the modal value.
c. NT, not tested.
d. Five VRSA strains with vancomycin MIC, >32 mg/L.

- When linezolid was combined with the other antimicrobials, the most favorable interaction was obtained with rifampin (66.7% partial synergy and 27.8% additive). All isolates showed an indifferent effect when linezolid was combined with gentamicin (Table 3).
- Vancomycin combinations showed predominantly additive (16.7 – 38.9%) or indifferent interactions (44.4 – 77.8%; Table 3).

Table 2. Distribution of isolates according to MBC/MIC ratios for daptomycin, vancomycin and linezolid.

	Number of strains with MBC/MIC ratios at:					
Organism (no. tested)	1	2	4	8	≥16[a]	≥32
MRSA-WT (101)						
Daptomycin	80	20	1	0	-	0
Linezolid	0	0	1	0	-	100
Vancomycin	40	21	12	13	8[a]	7
hVISA (64)						
Daptomycin	49	15	0	0	-	0
Linezolid	0	0	0	0	-	64
Vancomycin	10	4	3	3	42[a]	2
VISA (37)						
Daptomycin	28	9	0	0	-	0
Linezolid	0	2	0	0	-	35
Vancomycin	2	1	0	2	30[a]	2
VRSA (5)[b]						
Daptomycin	4	1	0	0	-	0
Linezolid	0	0	0	0	-	5

a. Isolates with MBC/MIC ratios ≥16 and MBC values ≥16 mg/L, the CLSI resistant breakpoint for vancomycin [CLSI, 2007].
b. MBC was not performed on the five VRSA strains.

Table 3. Summary of checkerboard synergy results by interaction category when testing 18 *S. aureus* strains.[a]

	No. of strains (%)				
Antimicrobial combinations	Synergy	Partial synergy	Additive	Indifferent	Antagonism
Daptomycin x gentamicin	0 (0.0)	9 (50.0)	5 (27.8)	4 (22.2)	0 (0.0)
Daptomycin x linezolid	0 (0.0)	1 (5.6)	5 (27.8)	12 (66.7)	0 (0.0)
Daptomycin x rifampin	0 (0.0)	2 (11.1)	4 (22.2)	12 (66.7)	0 (0.0)
Daptomycin x vancomycin	0 (0.0)	5 (27.8)	3 (16.7)	10 (55.6)	0 (0.0)
Linezolid x gentamicin	0 (0.0)	0 (0.0)	0 (0.0)	18 (100.0)	0 (0.0)
Linezolid x rifampin	0 (0.0)	12 (66.7)	5 (27.8)	1 (5.6)	0 (0.0)
Linezolid x vancomycin	0 (0.0)	1 (5.6)	3 (16.7)	14 (77.8)	0 (0.0)
Vancomycin x gentamicin	0 (0.0)	3 (16.7)	7 (38.9)	8 (44.4)	0 (0.0)
Vancomycin x rifampin	0 (0.0)	2 (11.1)	4 (22.2)	12 (66.7)	0 (0.0)

a. Fifteen strains were MRSA and three were MSSA, including the *S. aureus* ATCC 29213 control strain.

CONCLUSIONS

- Daptomycin was highly bactericidal against *S. aureus* strains and its bactericidal activity was not affected by decreased susceptibility to vancomycin.
- Linezolid showed bacteriostatic activity (elevated MBC) against the vast majority of *S. aureus* strains (98.6%), independent of their susceptibility to vancomycin.
- A worrisome proportion of MRSA-WT (14.9%) and the majority of hVISA (68.8%) and VISA (86.5%) strains showed vancomycin MBC/MIC ratios consistent with tolerance.
- The combinations of daptomycin with gentamicin and linezolid with rifampin generally exhibited a partial synergy or additive interaction by the checkerboard methods; while indifferent and additive interactions predominated among all other combinations evaluated.
- None of the combinations evaluated demonstrated antagonism.

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