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Inhibitory Activities of 11 Antimicrobial Agents and Bactericidal Activities of Vancomycin and Daptomycin against Invasive Methicillin-Resistant *Staphylococcus aureus* Isolates Obtained from 1999 through 2006▽

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We assessed MICs and minimal bactericidal concentrations of vancomycin, daptomycin, and nine other antimicrobials against methicillin-resistant *Staphylococcus aureus* isolates obtained from 1999 through 2006. No vancomycin, daptomycin, or linezolid resistance was observed. Clindamycin, gentamicin, and ciprofloxacin resistance decreased significantly. No tolerance to vancomycin or daptomycin was observed, nor was MIC creep seen.

The increasing prevalence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has been accompanied by debate over the effectiveness of vancomycin, perhaps due to diminished inhibitory or bactericidal activity that may have occurred in recent years. Some recent studies have differed with respect to the stability of vancomycin MICs over time (5, 8, 9, 18, 21). An earlier study from University of Texas Health Science Center and University Hospital indicated that the activity of vancomycin against MRSA did not change between the years 1987 and 1999, during a period of increasing resistance to other drug classes (e.g., macrolides, lincosamides, and fluoroquinolones) (9).

The presence and/or significance of antimicrobial tolerance remains an area of controversy (15, 20). Vancomycin tolerance was not related to outcome in an animal model of *S. aureus* endocarditis (20). However, reduced bactericidal activity of vancomycin (using a high-inoculum method) correlated with worse outcome in bacteremic patients treated with vancomycin (15).

The purpose of this study was to assess the inhibitory and bactericidal activities of vancomycin and daptomycin and inhibitory activities of nine other commonly used antimicrobial agents against isolates of MRSA recovered from bacteremic patients at a university hospital during an 8-year period from 1999 through 2006.

The first 30 MRSA isolates recovered from bacteremic patients each year were retrieved from the frozen isolate bank of the University Hospital Microbiology Laboratory during the years 1999 through 2006. Vancomycin troughs have been carefully monitored for approximately the last 2 years of the study period. These strains represented the first isolate recovered from each patient (not posttherapy), had been stored in skim milk at −70°C since isolation, and had been subcultured a minimum of two times.

Isolates were tested by the CLSI (formerly NCCLS) broth microdilution procedure (3); the test medium was cation-adjusted Mueller-Hinton broth. Panels incorporated 1/2-$\log_2$ dilutions of vancomycin (range of concentrations, 0.12 to 8 μg/ml). Also incorporated were standard dilutions of daptomycin (0.12 to 8 μg/ml, with 50 μg/ml calcium added to each daptomycin well), linezolid (0.06 to 16 μg/ml), erythromycin (0.06 to 64 μg/ml), clindamycin (0.03 to 16 μg/ml), doxycycline (0.06 to 16 μg/ml), minocycline (0.06 to 16 μg/ml), trimethoprim-sulfamethoxazole (0.03 to 8 μg/ml), gentamicin (0.06 to 8 μg/ml), rifampin (0.25 to 8 μg/ml), and ciprofloxacin (0.06 to 16 μg/ml). Microdilution panels were incubated at 35°C in air for 20 to 22 h prior to visual determination of MICs. Erythromycin-resistant isolates were tested for inducible resistance by the D-zone method (4).

Minimal bactericidal concentration (MBC) testing with vancomycin and daptomycin was performed for the first 10 isolates of each year; bactericidal effect (MBC) was defined as a 99.9% reduction in the initial inoculum density (14). Antimicrobial tolerance was defined as an MBC/MIC ratio of greater than or equal to a 5-$\log_2$-concentration difference (ratio of ≥32).

The range of MICs, MIC_{50}, MIC_{90}, MBC_{50}, and MBC_{90} and the percentage of isolates resistant to each agent were determined for each year. Statistical analyses, including chi-square and Fisher's exact tests, were performed using SPSS version 13 (SPSS, Inc., Chicago, IL).

All 240 isolates were susceptible to vancomycin, daptomycin, and linezolid; no isolate exhibited a vancomycin MIC exceeding 1.5 μg/ml (Table 1). For each year, the respective MIC_{50} and MIC_{90} of vancomycin were 0.75 and 0.75 μg/ml; those of daptomycin were 0.5 and 0.5 μg/ml; and those of linezolid were either 2 and 4 μg/ml or 4 and 4 μg/ml.

Clindamycin constitutive and inducible resistance rates decreased significantly during the study period. Erythromycin resistance remained 90 to 100% for every year included. Resistance to doxycycline decreased from 13.3 to 0% (not signif-

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TABLE 1. Summary of antimicrobial agent susceptibilities reported as MIC_{50} and MIC_{90}, MIC range, percent resistant by year, and MBC_{50} and MBC_{90}

Yr isolate obtained and antimicrobial agent[a]	MIC (μg/ml)[b]			% of isolates resistant	MBC_{50} (=MBC_{90} [μg/ml])[c]
	50%	90%	Range		
1999					
Vancomycin	0.75	0.75	0.38–0.75	0	0.75
Daptomycin	0.5	0.5	0.25–0.5	0	0.5
Clindamycin	>8	>8	0.06–>8	83.3	NP
Doxycycline	0.25	8	0.12–>16	13.3	NP
Minocycline	0.06	0.5	0.06–4	0	NP
Trim-Sulfa	0.12	4	0.06–>8	10	NP
Ciprofloxacin	>16	>16	0.25–>16	90	NP
Linezolid	4	4	2–4	0	NP
Gentamicin	2	>8	0.5–>8	30	NP
Rifampin	0.25	0.25	0.25–>8	3.3	NP
Erythromycin	>64	>64	0.5–>64	93.3	NP
2000					
Vancomycin	0.75	0.75	0.38–1.5	0	0.75
Daptomycin	0.5	0.5	0.25–1	0	0.5
Clindamycin	>8	>8	0.12–>8	83.3	NP
Doxycycline	0.25	8	0.12–8	10	NP
Minocycline	0.06	0.5	0.06–>8	6.7	NP
Trim-Sulfa	0.12	4	0.06–>8	10	NP
Ciprofloxacin	>16	>16	0.25–>16	93.3	NP
Linezolid	4	4	2–4	0	NP
Gentamicin	1	>8	0.5–>8	26.7	NP
Rifampin	0.25	0.25	0.25–2	3.3	NP
Erythromycin	>64	>64	0.5–>64	96.7	NP
2001					
Vancomycin	0.75	0.75	0.38–1.5	0	0.75
Daptomycin	0.5	0.5	0.25–0.5	0	0.5
Clindamycin	>8	>8	0.12–>8	60	NP
Doxycycline	0.25	0.5	0.12–8	3.3	NP
Minocycline	0.06	0.12	0.06–4	0	NP
Trim-Sulfa	0.12	0.12	0.06–>8	6.7	NP
Ciprofloxacin	>16	>16	0.25–>16	70	NP
Linezolid	4	4	2–4	0	NP
Gentamicin	1	4	0.5–>8	6.7	NP
Rifampin	0.25	0.25	0.25–1	0	NP
Erythromycin	>64	>64	0.25–>64	90	NP
2002					
Vancomycin	0.75	0.75	0.38–0.75	0	0.75
Daptomycin	0.5	0.5	0.25–1	0	0.5
Clindamycin	>8	>8	0.12–>8	66.7	NP
Doxycycline	0.25	2	0.12–4	3.3	NP
Minocycline	0.06	0.25	0.06–1	0	NP
Trim-Sulfa	0.12	0.12	0.06–2	0	NP
Ciprofloxacin	>16	>16	0.25–>16	86.7	NP
Linezolid	4	4	2–4	0	NP
Gentamicin	1	2	0.25–>8	0	NP
Rifampin	0.25	0.25	0.25–0.25	0	NP
Erythromycin	>64	>64	>64–>64	100	NP
2003					
Vancomycin	0.75	0.75	0.38–0.75	0	0.75
Daptomycin	0.5	0.5	0.25–1	0	0.5
Clindamycin	0.25	>8	0.12–>8	46.7	NP
Doxycycline	0.25	0.25	0.12–>16	3.3	NP
Minocycline	0.06	0.12	0.12–>16	3.3	NP
Trim-Sulfa	0.12	0.25	0.06–>8	3.3	NP
Ciprofloxacin	16	>16	0.25–>16	60	NP
Linezolid	4	4	2–4	0	NP
Gentamicin	1	2	0.5–>8	6.7	NP
Rifampin	0.25	2	0.25–>8	10	NP
Erythromycin	>64	>64	>64–>64	100	NP

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TABLE 1—*Continued*

Yr isolate obtained and antimicrobial agent[a]	MIC (μg/ml)[b]			% of isolates resistant	MBC_{50} (=MBC_{90} [μg/ml])[c]
	50%	90%	Range		
2004					
Vancomycin	0.75	0.75	0.38–0.75	0	0.75
Daptomycin	0.5	0.5	0.25–1	0	0.5
Clindamycin	>8	>8	0.12–>8	53.3	NP
Doxycycline	0.25	0.5	0.25–4	0	NP
Minocycline	0.06	0.06	0.06–0.12	0	NP
Trim-Sulfa	0.12	0.12	0.06–1	0	NP
Ciprofloxacin	16	>16	0.25–>16	73.3	NP
Linezolid	2	4	1–4	0	NP
Gentamicin	1	2	0.12–2	0	NP
Rifampin	0.25	0.25	0.25–0.25	0	NP
Erythromycin	>64	>64	0.25–>64	100	NP
2005					
Vancomycin	0.75	0.75	0.38–0.75	0	0.75
Daptomycin	0.5	0.5	0.25–1	0	0.5
Clindamycin	0.25	>8	0.12–>8	30	NP
Doxycycline	0.25	1	0.12–2	0	NP
Minocycline	0.06	0.12	0.06–0.5	0	NP
Trim-Sulfa	0.12	1	0.06–2	0	NP
Ciprofloxacin	16	>16	0.25–>16	83.3	NP
Linezolid	4	4	1–4	0	NP
Gentamicin	0.5	2	0.5–>8	6.7	NP
Rifampin	0.25	0.25	0.25–0.5	0	NP
Erythromycin	>64	>64	16–>64	100	NP
2006					
Vancomycin	0.75	0.75	0.38–0.75	0	0.75
Daptomycin	0.5	0.5	0.5–0.5	0	0.5
Clindamycin	0.25	>8	0.12–>8	16.7	NP
Doxycycline	0.25	1	0.12–4	0	NP
Minocycline	0.06	0.12	0.06–0.5	0	NP
Trim-Sulfa	0.12	1	0.06–>8	3.3	NP
Ciprofloxacin	16	>16	0.25–>16	63.3	NP
Linezolid	2	4	2–4	0	NP
Gentamicin	0.5	1	0.5–2	0	NP
Rifampin	0.25	0.25	0.25–8	3.3	NP
Erythromycin	>64	>64	0.25–>64	90	NP

[a] Trim-Sulfa, trimethoprim-sulfamethoxazole tested at a 1:19 ratio. (MICs reflect the trimethoprim component.)
[b] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[c] NP, not performed.

icant), resistance to gentamicin decreased from 30 to 0% ($P < 0.001$), and resistance to ciprofloxacin decreased from 90 to 63% ($P < 0.01$). Susceptibility rates to trimethoprim-sulfamethoxazole, rifampin, doxycycline, and minocycline remained high throughout the study period.

Tolerance to vancomycin or daptomycin was not observed: the MIC_{50} and MIC_{90} equaled the MBC_{50} and MBC_{90}, respectively, for both vancomycin and daptomycin.

This study found that antimicrobial activities of several agents against invasive isolates of MRSA during the recent 8-year period varied over time; however, vancomycin and daptomycin activities remained stable in terms of inhibitory and bactericidal effects.

Vancomycin is a widely accepted agent for the treatment of invasive MRSA infections (17, 19). However, vancomycin has been associated with slower clinical response to therapy of *S. aureus* compared with other cell-wall-active agents, i.e., β-lactams (2, 10, 16). Vancomycin treatment failures have called into question the effectiveness of this agent in the therapy of bacteremia, as well as lower respiratory tract infections (6, 7, 12).

Explanations for suboptimal clinical response have included "MIC creep," a subtle increase in vancomycin MIC (within susceptible range) over time. Our results support the stability of vancomycin MICs over time as previously demonstrated (5, 8, 9). Although higher vancomycin MICs within the susceptible range have been associated with worse treatment outcomes in some studies (6, 7, 13), we report that the MIC_{90} of vancomycin remained under 1 μg/ml during each year studied. The highest vancomycin MIC noted in this study, 1.5 μg/ml (identified once each in 2000 and 2001), would have been reported using a standard dilution panel as 2 μg/ml, a level at which some authors have raised concern (13, 15).

No daptomycin- or linezolid-nonsusceptible isolates were found during the present investigation, and there was no notable increase in their MICs during the study period, which included the time before the drugs were available for clinical use and the period in which they had been prescribed in our institution.

Study limitations include geographical restriction to a single institution, the limited number of isolates sampled, and lack of correlating clinical information. Only one method was used to measure MIC/MBC, and no strategy was employed to evaluate strains for heteroresistance (22). These strains were pretherapy isolates and had been subcultured a minimum of two times, which may affect the ability to demonstrate MIC increases over time (1). Our selection method, however, should reflect a shift toward higher vancomycin MICs if this were to occur.

Epidemiology from our institution has mirrored the shift from hospital-associated MRSA types (e.g., USA100) to a significant proportion of CA-MRSA types (primarily USA300) over time (11), and this likely explains the decline in rates of resistance to clindamycin, ciprofloxacin, and gentamicin. This study of MRSA isolates from bacteremic patients during the most recent 8-year period has demonstrated susceptibility to vancomycin, daptomycin, and linezolid; stability of vancomycin MICs over time; and increasing susceptibility to several other drug classes. In one of the largest investigations of bactericidal activity in contemporary MRSA clinical isolates, we found no evidence of vancomycin or daptomycin tolerance.

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