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Inhibitory and Bactericidal Activities of Daptomycin, Vancomycin, and Teicoplanin against Methicillin-Resistant *Staphylococcus aureus* Isolates Collected from 1985 to 2007[▽]

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The inhibitory and bactericidal activities of daptomycin, vancomycin, and teicoplanin against a collection of 479 methicillin-resistant *Staphylococcus aureus* isolates were assessed. The isolates were collected from U.S. and European hospitals from 1985 to 2007 and were primarily from blood and abscess cultures. The MICs and minimum bactericidal concentrations (MBCs) of the three agents were determined, and the MBC/MIC ratios were calculated to determine the presence or absence of tolerance. Tolerance was defined as an MBC/MIC ratio of ≥32 or an MBC/MIC ratio of ≥16 when the MBC was greater than or equal to the breakpoint for resistance. Tolerance to vancomycin and teicoplanin was observed in 6.1% and 18.8% of the strains, respectively. Tolerance to daptomycin was not observed.

Although vancomycin and teicoplanin are the standard therapies for staphylococcal bacteremia, tolerance to vancomycin and teicoplanin has been demonstrated in both coagulase-negative staphylococci and *Staphylococcus aureus* as well as in various *Streptococcus* species (2, 3, 7, 10, 13, 15, 20, 21, 23, 25). Daptomycin, a lipopeptide antibiotic, has been demonstrated to have rapid bactericidal activity against gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), and tolerance to this drug has not been demonstrated (2, 9, 10, 19, 21, 24, 26, 28).

The issue of antibiotic tolerance is a complicated one. Some studies have suggested that infections caused by tolerant strains may be more difficult to treat, especially when they cause complicated infections such as endocarditis, meningitis, or osteomyelitis or cause infections in immunocompromised patients (7, 8, 14, 15, 16, 18, 20, 22, 23, 25). Other investigators' expert analyses do not agree that there is proof of a correlation between tolerant strains and treatment failures or that bactericidal activity is required for the treatment of serious MRSA infections (17, 25, 26, 27, 28). Controversy concerning the appropriate methods for the determination of tolerance in clinical isolates and in the practicality of testing isolates for tolerance in the clinical laboratory also exists.

This study looked at MRSA isolates obtained primarily from blood and abscess cultures collected between 1985 and 2007. The main purpose of the study was to determine the in vitro inhibitory and bactericidal activities and the level of tolerance to the three drugs observed by standardized MIC and minimum bactericidal concentration (MBC) tests (4, 5, 19).

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MATERIALS AND METHODS

Microorganisms. A collection of 479 MRSA isolates, primarily from blood and abscess sources of infection, collected from U.S. and European hospitals between 1985 and 2007 were tested. The isolates were unique and nonconsecutive and were collected for a variety of studies. All of the strains were susceptible to daptomycin, vancomycin, and teicoplanin and resistant to oxacillin by previous MIC testing. Vancomycin-intermediate and -resistant strains were excluded from the test set.

Susceptibility testing. MICs were determined by broth microdilution, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical and Laboratory Standards) (5, 6). Daptomycin (Cubist Pharmaceuticals), vancomycin (Sigma-Aldrich), and teicoplanin (Molcan Corp) were tested at dilution ranges of 0.015 to 512 μg/ml. Standard cation-adjusted Mueller Hinton Broth (CAMHB) was used for vancomycin and teicoplanin MIC testing. CAMHB containing 50 mg/liter of calcium and 11 mg/liter of magnesium was used for daptomycin MIC testing. Quantitative colony counts were performed from the growth control well of each microdilution panel.

MBCs were determined in accordance with the guidelines of the Clinical and Laboratory Standards Institute (4) and the work of Peterson and Shanholtzer (19). The entire volume (0.1 ml) of the MIC well and the wells with 4 dilutions above the MIC were spread across the center of a blood agar plate and allowed to dry for 20 min. After the plates had dried, a sterile spreading rod was used to evenly disperse the inoculum over the entire surface of the plate, which was then incubated at 35 to 37°C for 24 to 48 h. The MBCs were recorded as the lowest dilution that produced a ≥99.9% reduction in growth (≥3-$\log_{10}$ reduction in CFU/ml) in comparison to the growth of the control.

RESULTS

Daptomycin was the most potent antibiotic evaluated, as determined from the MIC_{50} and the MIC_{90} (0.5 and 0.5 μg/ml, respectively); vancomycin and teicoplanin had MIC_{50}s of 1 and 1 μg/ml, respectively, and MIC_{90}s of 0.5 and 1 μg/ml, respectively (Table 1). Daptomycin was also highly bactericidal, as determined from its MBC_{50} and MBC_{90} (0.5 and 1 μg/ml), followed by vancomycin and teicoplanin, for which the MBC_{50}s were 1 and 2 μg/ml, respectively, and the MBC_{90}s were 1 and 32 μg/ml, respectively (Table 1).

Tolerance, defined as an MBC/MIC ratio of ≥32 or an MBC/MIC ratio of ≥16 with an MBC in the resistant range, was evaluated (2, 13). The overall tolerance rates for vancomycin and teicoplanin were 6.1% (29/479 isolates) and 18.8%

TABLE 1. MIC_{50}s, MIC_{90}s, MBC_{50}s, MBC_{90}s, and percent tolerance for 479 isolates collected from 1985 to 2007

Drug[a]	MIC_{50}/MIC_{90} (μg/ml)	MBC_{50}/MBC_{90} (μg/ml)	% Tolerance[b]
DAP	0.5/0.5	0.5/1	0
VAN	1/1	1/2	6.1
TEI	0.5/1	1/32	18.8

[a] DAP, daptomycin; VAN, vancomycin; TEI, teicoplanin.
[b] Percent tolerance was defined as an MBC/MIC ratio of ≥32 or an MBC/MIC ratio of ≥16 when the MBC was greater than or equal to the breakpoint for resistance.

(90/479 isolates), respectively. No tolerance was observed for daptomycin (Tables 1 and 2). Vancomycin MBC/MIC ratios were 1 for 84.5% of the strains and ≤2 for 92.5% of the strains, while the teicoplanin MBC/MIC ratios were 1 for 49.3% of the strains and ≤2 for 74.3% of the strains (Table 2). Daptomycin MBC/MIC ratios were 1 for 87.5% of the strains and ≤2 for 100% of the strains (Table 2).

The MICs for vancomycin and teicoplanin were not predictive of tolerance in this study, similar to the findings of a study of strains from 30 bacteremic patients reported by Sakoulas et al. (23). The incidence of tolerance at each MIC increased as the MIC increased (Tables 3 and 4). Among the isolates in the group of 29 vancomycin-tolerant isolates, 24 (82.8%) were also tolerant to teicoplanin (Table 5). The highest daptomycin MIC in this group was 1 μg/ml (one isolate), and two isolates had MBC/MIC ratios of 2 (data not shown).

Twenty-four of 90 teicoplanin-tolerant isolates (26.7%) were also vancomycin tolerant (Table 6). Among the isolates in this group, 12 (15.4%) isolates had daptomycin MICs of 1 μg/ml, while the other 78 strains had daptomycin MICs of ≤0.5 μg/ml. Twelve of the strains tolerant to teicoplanin had daptomycin MBC/MIC ratios of only 2 (data not shown).

DISCUSSION

Since tolerance to daptomycin was not exhibited in vitro in this study or in other studies, it has been suggested that daptomycin remains an alternative to standard therapy for bacteremia, endocarditis, and other life-threatening infections caused by *S. aureus* (2, 8, 9, 10, 14, 26, 27). For vancomycin, studies looking at tolerance by the use of MBC or time-kill studies have shown a variety of results. The rates of tolerance to vancomycin have ranged from 0% (12) to as high as 47% (15). Biedenbach et al. (2) reported a 3.2% rate of tolerance to vancomycin and a 31.6% rate of tolerance to teicoplanin for 76 MRSA isolates from a collection of SENTRY Antimicrobial Surveillance Program strains collected from eight medical centers in the Asia-Pacific region, while Jones (13) reported a rate of tolerance to vancomycin of 15% for 105 wild-type MRSA isolates collected from medial centers across six continents for inclusion in the 1997–2003 SENTRY Antimicrobial Surveillance Program database. There are fewer data in the literature on tolerance to teicoplanin; however, isolates have been shown to exhibit more tolerance to teicoplanin than to vancomycin in previous studies (2, 15).

The issue of tolerance on the basis of bactericidal test results remains complicated, since technical factors have been known to affect the results and tolerance measured in vitro may not always translate to tolerance in vivo (4, 17, 21). The two types of tests used to determine the presence of tolerance are the MBC and the time-kill assays. Both tests use the same definition of bactericidal activity: a reduction of the CFU by 99.9% (≥3-log_{10}-unit reduction in the numbers of CFU/ml) from the starting inoculum. Isolates are considered tolerant to antimicrobial agents that are known to be bactericidal but that do not show killing at an MBC/MIC ratio of ≥32 or an MBC/MIC ratio of ≥16 with an MBC above the MIC resistance breakpoint (4, 9, 13, 15, 18, 19). May et al. (15) found the two methods to be comparable. The MBC test has been standard-

TABLE 2. MBC-to-MIC ratios for daptomycin, vancomycin, and teicoplanin across all study years

MBC/MIC ratio	No. (%) of isolates with the indicated MBC/MIC ratio for[a]:		
	Daptomycin	Vancomycin	Teicoplanin
1	419 (87.5)	405 (84.6)	236 (49.3)
2	60 (12.5)	38 (7.9)	120 (25.1)
4	0	2 (0.4)	25 (5.2)
8	0	4 (0.8)	3 (0.6)
≥16	**0**	**3 (0.6)**	**10 (2.1)**
≥32	**0**	**27 (5.6)**	**85 (17.7)**

[a] The MBC/MIC ratios of the three drugs for all isolates were determined. Boldface data are for tolerant strains.

TABLE 3. Evidence of vancomycin tolerance at each MIC

MIC (μg/ml)	No. of isolates[a]		% Tolerant at MIC
	Tolerant	With the indicated MIC	
≤0.25	0	2	0.0
0.5	1	120	0.8
1	26	348	7.5
2	2	7	28.6
4	**0**	**2**	**0.0**
8	**0**	**0**	**0.0**
≥16	**0**	**0**	**0.0**
Total	29	479	6.1

[a] Tolerance was defined as an MBC-to-MIC ratio of ≥32 or an MBC-to-MIC ratio of ≥16 and an MBC of ≥16 (μg/ml). Boldface data are for isolates that are intermediate or resistant according to their MICs, in accordance with CLSI guidelines.

TABLE 4. Evidence of teicoplanin tolerance at each MIC

MIC (μg/ml)	No. of isolates[a]		% Tolerant at MIC
	Tolerant	With the indicated MIC	
≤0.25	5	55	9.1
0.5	29	304	9.5
1	38	96	39.6
2	12	17	70.5
4	4	5	80.0
8	1	1	100.0
≥16	**1**	**1**	**100.0**
Total	90	479	18.8

[a] Tolerance is defined as an MBC-to-MIC ratio of ≥32 or MBC-to-MIC ratio of ≥16 and an MBC of ≥32 μg/ml. Boldface data are for isolates that are intermediate or resistant according to their MICs, in accordance with CLSI guidelines.

TABLE 5. Comparative MBC/MIC ratios of 29 vancomycin-tolerant strains

Drug (MBC/MIC ratio)	No. of strains	% of strains
Daptomycin		
1	27	93.1
2	2	6.9
Teicoplanin		
2	4	13.8
16	1	3.4
Tolerant[a]	24	82.8

[a] MBC/MIC ratio of ≥32 or MBC/MIC ratio of ≥16 and MBC of ≥32 μg/ml.

TABLE 6. Comparative MBC/MIC ratios of 90 teicoplanin-tolerant strains

Drug (MBC/MIC ratio)	No. of strains	% of strains
Daptomycin		
1	76	84.4
2	14	15.6
Vancomycin		
1	52	57.8
2	10	11.1
4	2	2.2
8	1	1.1
16	1	1.1
Tolerant[a]	24	26.7

[a] MBC/MIC ratio of ≥32 or MBC/MIC ratio of ≥16 and MBC of ≥16 μg/ml.

ized, can be performed in the clinical laboratory, and, if it is done correctly, has been shown to be reproducible (4, 11, 19). Because of the relative ease of performance, the MBC test can be one of the tools that clinicians can use to make treatment decisions for individual patients (11, 19).

Conclusions. Daptomycin was more potent in vitro than either vancomycin or teicoplanin against MRSA according to its MIC_{90} and was more bactericidal according to its MBC_{90} and MBC/MIC ratios. A total of 6.1% (29/479) and 18.8% (90/479) of the strains tested exhibited tolerance to vancomycin and teicoplanin, while tolerance to daptomycin was not observed for any of the 479 isolates. Twenty-four (5%) of all strains were tolerant to both vancomycin and teicoplanin. There have been a variety of rates of vancomycin and teicoplanin tolerance reported in the literature; one explanation for the variability might be that there has been a wide variety in the organism sets that were studied. In addition there has been variability in the MBC and/or time-kill methods used to determine tolerance rates. Finally, the MICs of vancomycin and teicoplanin were not predictive of tolerance; however, the percentage of strains exhibiting tolerance increased as the MICs of both vancomycin and teicoplanin increased in this study.

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