

Comparison of the Agar Dilution, Tube Dilution, and Broth Microdilution Susceptibility Tests for Determination of Teicoplanin MICs

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The purpose of this study was to evaluate the National Committee for Clinical Laboratory Standards agar dilution, tube dilution, and broth microdilution susceptibility tests for the measurement of teicoplanin MICs. The three standardized tests gave equivalent (within a twofold dilution) results with 98.8 to 99.0% of the 508 gram-positive clinical isolates tested, indicating that either method may be used for teicoplanin MIC determination.

Teicoplanin (Targocid) is a glycopeptide antibiotic active against gram-positive bacteria. The MICs for 90% of a variety of organisms, as measured by the agar dilution, tube broth dilution, or broth microdilution susceptibility test, range from 0.008 to 4.0 $\mu\text{g/ml}$ (2, 3, 5-8). In this study, we directly compared these three techniques for measuring teicoplanin MICs.

The assay quality control organisms *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were purchased from Difco Laboratories, Detroit, Mich. (Bactrol MIC set). The 508 gram-positive clinical isolates tested were obtained from patients with skin and soft tissue infections, vascular and nonvascular access-associated bacteremias or septicemias, drug abuse-associated endocarditis, and those with acute bone and joint infections. A total of 39 investigators at 38 different clinical sites supplied those cultures. The identities of all isolates were confirmed in our laboratory. The isolates included 2 *Micrococcus luteus*, 265 *S. aureus*, 103 *Staphylococcus epidermidis*, 11 *Staphylococcus haemolyticus*, 2 *Staphylococcus cohnii*, 3 *Staphylococcus capitis*, 6 *Staphylococcus hominis*, 1 *Staphylococcus warneri*, 1 *Staphylococcus saprophyticus*, and 2 *Staphylococcus simulans* strains; 56 beta-hemolytic streptococci; 15 viridans group streptococci; 2 *Streptococcus pneumoniae* strains; 32 enterococci; 1 unidentified *Leuconostoc* sp.; 5 unidentified diphtheroids; and 1 unidentified *Bacillus* sp.

The susceptibility tests were performed in accordance with the procedures outlined by the National Committee for Clinical Laboratory Standards (4). For each test, isolates were tested simultaneously by the three test formats with teicoplanin test dilutions derived from a freshly prepared (0.1 M sodium phosphate buffer, pH 7.4) common master stock solution.

The data from the comparative susceptibility tests are summarized in Table 1. The three test formats gave comparable (within a twofold dilution of equivalence) results for 98.8% (502 of 508 for agar dilution versus tube dilution and agar dilution versus broth microdilution) to 99.0% (503 of 508 for broth microdilution versus tube dilution) of the clinical isolates evaluated. The geometric mean MICs for all strains evaluated were 1.05, 1.02, and 1.05 $\mu\text{g/ml}$ for the agar dilution, broth microdilution, and the tube dilution test

TABLE 1. Comparison of standardized susceptibility tests for determination of teicoplanin MICs ($n = 508$)

Test comparison ^a	No. of MICs higher at a doubling dilution difference of:				
	-2	-1	0	+1	+2
Broth microdilution vs agar dilution	4	125	300	77	2
Tube dilution vs agar dilution	2	156	279	67	4
Tube dilution vs broth microdilution	3	104	325	74	2

^a Each isolate was tested at teicoplanin concentrations of 0.03 to 16.0 $\mu\text{g/ml}$.

formats, respectively. However, overall agar dilution MICs tended to be slightly higher. For example, in the comparison of the agar dilution test with the broth microdilution assay, 79 (15.6%) of the values were higher by the microdilution method while 129 (25.4%) were higher by agar dilution. In the comparison of the agar dilution test with the tube dilution assay, 71 (13.9%) of the MICs were higher by the tube dilution method while 158 (31.1%) were higher by agar dilution. This skewing could not be attributed to any particular group of organisms. For example, the geometric mean MICs for all *S. aureus* strains were 0.93, 0.92, and 0.88 $\mu\text{g/ml}$ for the agar dilution, broth microdilution, and tube dilution test formats, respectively. Agar dilution, broth microdilution, and tube dilution geometric mean MICs for the other

TABLE 2. Quality control data for the 31 assays used to generate the comparative MICs

Organism	MIC ($\mu\text{g/ml}$)	No. of positive assays		
		Agar dilution	Broth microdilution ^a	Tube dilution
<i>S. aureus</i> ATCC 29213	1.0	10	4	3
	0.5	21	27	28
<i>E. faecalis</i> ATCC 29212	0.5	24	12	4
	0.25	7	18	27
	0.125	0	1	0

^a Tentative broth microdilution quality control limits (1), 0.12 to 0.5 μg of teicoplanin per ml for *S. aureus* and 0.06 to 0.25 μg of teicoplanin per ml for *E. faecalis*.

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groups of organisms tested were, respectively, the following: 2.16, 2.12, and 2.25 $\mu\text{g/ml}$ for the coagulase-negative staphylococci; 0.42, 0.38, and 0.31 $\mu\text{g/ml}$ for the enterococci; and 0.11, 0.09, and 0.08 $\mu\text{g/ml}$ for the streptococci. This trend was also mirrored in the quality control data generated during this study (Table 2).

In summary, the results presented here indicate that any one of the three National Committee for Clinical Laboratory Standards standardized susceptibility tests can be used to determine teicoplanin MICs.

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