

Evaluation of Teicoplanin and Vancomycin Disk Susceptibility Tests

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Disk tests with two glycopeptide antibiotics, teicoplanin and vancomycin, were evaluated, and MICs were compared with those of fusidic acid and coumermycin. For tests with 30- μ g vancomycin disks, we recommend modification of the current zone size standards to ≤ 10 mm for resistant and ≥ 15 mm for susceptible. For teicoplanin disk tests, 30- μ g disks are recommended, with zone size interpretive standards of ≤ 10 and ≥ 14 mm. Since no resistant clinical isolates are available at this time, susceptibility testing of either drug is rarely necessary, and zone size standards are tentative.

Teicoplanin (formerly teichomycin A2) is a complex of glycopeptide antibiotics related to vancomycin and ristocetin. The major components have molecular weights of about 1,900 (2). Teicoplanin interferes with cell wall synthesis by inhibiting polymerization of peptidoglycans (9). Like vancomycin, teicoplanin is bactericidal against gram-positive microorganisms (7, 8). It is approximately twice as active as vancomycin against *Staphylococcus* spp., including methicillin-resistant strains, and is distinctly more active than vancomycin against streptococci, including the enterococci (1, 3, 7, 8, 10). Teicoplanin is also active against the JK group of corynebacteria (4, 7), *Listeria monocytogenes* (7), and anaerobic, gram-positive bacteria (8). Fusidic acid and coumermycin are two other antimicrobial agents with similar spectrums of activity largely limited to gram-positive bacteria, especially methicillin-resistant and -susceptible staphylococci.

The purpose of this report is to describe our efforts to evaluate teicoplanin disk susceptibility tests and to reevaluate vancomycin disk test interpretive standards. In vitro data comparing teicoplanin with vancomycin, fusidic acid, and coumermycin are also reported.

MATERIALS AND METHODS

Antibiotics. Teicoplanin was kindly provided by Merrell Dow Pharmaceuticals, Inc. (Cincinnati, Ohio). At the time it was prepared, the powder was reported to contain approximately 78% A2 and 6% A3 components as defined by high-pressure liquid chromatography. Other comparative drugs included vancomycin (Eli Lilly & Co., Indianapolis, Ind.), fusidic acid (Leo Pharmaceutical Products, Copenhagen, Denmark), and coumermycin (Hoffman-La Roche, Inc., Nutley, N.J.). Vancomycin (30 μ g) disks were obtained from Difco Laboratories (Detroit, Mich.) (lot no. 717325), and disks containing 15, 30, or 60 μ g of teicoplanin were prepared at the Clinical Microbiology Institute (Tualatin, Oregon).

Antimicrobial susceptibility tests. Microdilution susceptibility tests were performed by the procedure outlined by the National Committee for Clinical Laboratory Standards (5). The inoculum was approximately 5×10^5 CFU/ml, and MICs were read after 16 to 18 h of incubation at 35°C in

ambient air, unless added CO₂ was needed for growth of the test strain. Cation-supplemented Mueller-Hinton medium was used throughout; 3% lysed horse blood was added for testing nonenterococcal streptococci and the JK group of corynebacteria. The addition of lysed horse blood did not alter the results of tests with the standard control strains of *Staphylococcus aureus* ATCC 29213 or *Streptococcus faecalis* ATCC 29212. Disk diffusion tests were also performed as outlined by the National Committee for Clinical Laboratory Standards (6). The medium was supplemented with 5% defibrinated sheep blood, if necessary for growth of the test strain. Additional blood did not influence the results of diffusion tests with the control strains.

Microorganisms. Selected clinical isolates were obtained from the stock culture collections of our laboratories. The 285 isolates included 105 *Staphylococcus aureus* (53 methicillin resistant), 28 coagulase-negative staphylococci (11 methicillin resistant), 11 *Corynebacterium* spp. of the JK group, 117 *Streptococcus* spp., and 24 gram-negative bacilli representing the following species: *Acinetobacter calcoaceticus* subsp. *anitratus*, *Citrobacter diversus*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, *Providencia stuartii*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, and *Pseudomonas maltophilia*.

RESULTS

In vitro activity. Table 1 summarizes the results of the microdilution tests with gram-positive microorganisms. All four drugs were inactive against the 24 representative gram-negative bacilli that were tested (data not shown). Against the staphylococci, teicoplanin was two to four times more active than vancomycin. Fusidic acid was 2 to 4 times more active than teicoplanin, and coumermycin was about 16 times more active than fusidic acid. Against the streptococci, teicoplanin was the most active compound, and fusidic acid was the least active drug that was tested. All four drugs effectively inhibited the JK group of corynebacteria.

Disk diffusion tests. The effect of increasing the potency of teicoplanin disks is described by the data in Table 2. Although teicoplanin was more active than vancomycin, 30- μ g teicoplanin disks produced zones 1 to 2 mm smaller than those around 30- μ g vancomycin disks. For each twofold

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TABLE 1. In vitro activity of teicoplanin, vancomycin, fusidic acid, and coumermycin against gram-positive microorganisms

Microorganism (no. tested)	MIC (mg/ml) for 50 and 90% of isolates in each group							
	Teicoplanin		Vancomycin		Fusidic acid		Coumermycin	
	50%	90%	50%	90%	50%	90%	50%	90%
<i>Staphylococcus aureus</i> ^a								
Pen S (27)	0.25	0.5	1.0	1.0	0.06	0.12	0.004	0.004
Pen R (25)	0.25	0.5	1.0	1.0	0.06	0.06	0.004	0.004
Meth R (53)	0.25	0.5	1.0	1.0	0.06	0.12	≤0.002	0.004
Coagulase-negative staphylococci ^a								
Pen S (10)	0.25	1.0	1.0	2.0	0.06	0.06	0.004	0.008
Pen R (18) ^b	1.0	2.0	1.0	2.0	0.06	0.06	0.004	0.008
<i>Corynebacterium</i> spp.								
JK group (11)	0.5	1.0	0.5	0.5	0.03	0.06	0.25	0.25
<i>Streptococcus</i> spp.								
<i>Streptococcus faecalis</i> (25)	0.12	0.25	2.0	4.0	4.0	8.0	1.0	4.0
Other enterococci (16) ^c	0.12	0.5	1.0	4.0	2.0	4.0	0.25	4.0
<i>Streptococcus agalactiae</i> (20)	0.12	0.25	0.5	0.5	8.0	8.0	0.25	0.5
<i>Streptococcus pyogenes</i> (20)	0.06	0.12	0.25	0.5	2.0	4.0	0.25	0.5
<i>Streptococcus pneumoniae</i> (20)	0.06	0.06	0.25	0.5	2.0	4.0	0.03	0.12
Other spp. (16) ^d	0.06	0.12	0.5	0.5	8.0	16.0	0.25	1.0

^a Susceptible (S) or resistant (R) to penicillin (Pen) or methicillin (Meth).
^b Includes 11 methicillin-resistant strains.
^c Includes 13 *Streptococcus faecium* and 3 *Streptococcus durans*.
^d Includes 6 *Streptococcus bovis*, 6 *Streptococcus mitis*, and 4 *Streptococcus sanguis*.

increase in teicoplanin disk potency, the average zone diameter increased by approximately 1 mm. Zones around 60-μg teicoplanin disks were comparable in size to those around 30-μg vancomycin disks.

Vancomycin disk tests. For purposes of this study, we categorized all strains with MICs ≤4.0 μg/ml as being susceptible to vancomycin, and strains with MICs >8.0 μg/ml were considered resistant. Only one strain (*Streptococcus faecium*) had an MIC of 8.0 μg/ml. All other gram-positive isolates were susceptible (MIC, ≤4.0 μg/ml), and the gram-negative bacilli were resistant (MIC, >16 μg/ml) to vancomycin.

Current National Committee for Clinical Laboratory Standards standards for the disk test (6) define zone size breakpoints of ≤9 mm for resistant and ≥12 mm for susceptible strains (MIC correlate, ≤5.0 μg/ml). With other antimicrobial agents, many problems have arisen when the resistant breakpoint is less than 10 mm and when the intermediate category is less than 3 mm. To avoid potential difficulties, we suggest modifying the interpretive breakpoints for tests with 30-μg vancomycin disks to ≤10 mm for resistant and ≥15 mm for susceptible strains (MIC correlate, ≤4.0 μg/ml). With that change, vancomycin disk

TABLE 2. Influence of teicoplanin disk potency on zone diameters with 133 staphylococci and 117 streptococci

Disk content (μg)	Mean (range) of zone diam (mm) for:	
	Staphylococci	Streptococci
Teicoplanin		
15	17.9 (14-26)	18.5 (14-26)
30	18.8 (14-27)	19.9 (15-28)
60	20.5 (16-30)	21.1 (16-30)
Vancomycin		
30	20.2 (17-30)	21.5 (16-31)

tests accurately categorized susceptible gram-positive cocci (Fig. 1).

Teicoplanin disk tests. The MIC interpretive breakpoints that were used for vancomycin were also used for tests with teicoplanin. With one exception, all of our strains were quite

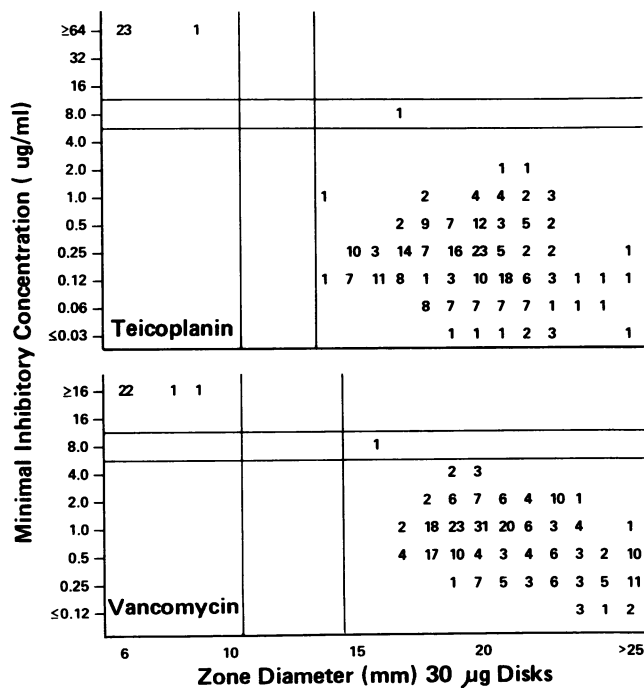


FIG. 1. Scattergrams showing correlations between vancomycin MICs and zone diameters around 30-μg vancomycin disks (bottom) and between teicoplanin MICs and zone diameters around 30-μg teicoplanin disks (top); numbers represent the number of data points at each location (285 isolates tested).

susceptible (MIC, ≤ 2.0 $\mu\text{g/ml}$) or very resistant (MIC, ≥ 128 $\mu\text{g/ml}$). The strain with intermediate susceptibility (MIC, 8.0 $\mu\text{g/ml}$) represents a methicillin-resistant coagulase-negative staphylococcus. That strain was retested and continued to be only moderately susceptible to teicoplanin (MIC, 8.0 $\mu\text{g/ml}$) but susceptible to vancomycin (MIC, 0.5 $\mu\text{g/ml}$).

Figure 1 displays the results of tests with 30- μg teicoplanin disks. Interpretive breakpoints of ≤ 10 and ≥ 14 mm would accurately predict susceptibility to teicoplanin. Only one minor discrepancy was observed with the moderately susceptible staphylococcus, which had an MIC of 8.0 $\mu\text{g/ml}$ but was susceptible with all three teicoplanin disk potencies.

Table 3 summarizes the results of tests with 15-, 30- and 60- μg teicoplanin disks. As noted above, a methicillin-resistant, coagulase-negative staphylococcus was moderately susceptible by MIC but was susceptible with all three disks. One resistant *Providencia stuartii* initially gave an 18-mm zone around the 60- μg disk, but when that strain was retested, there was a partial zone with definite growth up to the edge of the disk. A strain of *Citrobacter diversus* gave an 11-mm zone around the 60- μg disk, and a strain of *Escherichia coli* produced a 9-mm zone around the 30- μg disk. We concluded that the 60- μg teicoplanin disk may be too potent and may erroneously classify resistant gram-negative strains as susceptible. There was no apparent reason to consider use of the 15- $\mu\text{g/ml}$ disk. Although all three disks performed satisfactorily, we selected the 30- μg disk for routine use.

DISCUSSION

Teicoplanin and vancomycin are two glycopeptide antibiotics with similar spectrums of activity, which are limited to gram-positive microorganisms. However, teicoplanin is more active than vancomycin. The activity of teicoplanin against the enterococci, methicillin-resistant staphylococci, and the JK group of corynebacteria is particularly noteworthy, since those pathogens present serious therapeutic problems. Both glycopeptide antibiotics differ from fusidic acid and coumermycin, two other drugs with spectrums of activity largely limited to gram-positive bacteria. Bacterial resistance among normally susceptible species has not been documented.

Using agar dilution procedures, other investigators (1, 2, 10) have found that teicoplanin is only slightly more active than vancomycin. Bauernfeind and Petermuller (1) documented the fact that teicoplanin agar dilution MICs were significantly greater than those observed with broth microdilution tests (up to 32-fold higher). Our teicoplanin microdilution MICs were four- to eightfold lower than those previously reported with agar dilution procedures. However, our vancomycin microdilution MICs were essentially comparable to those reported in the previously cited studies. With either testing procedure, teicoplanin was more active than vancomycin; the methodology only influenced the magnitudes of the differences between the two drugs.

The unique pharmacokinetic properties of teicoplanin distinguish it from vancomycin (10, 11). Peak levels in serum are similar to those observed with vancomycin, but the elimination half-life of teicoplanin is extremely prolonged, i.e., in excess of 40 h (10, 11). With daily intravenous or intramuscular injection, the level in serum should always exceed 2.0 $\mu\text{g/ml}$. At half the dosing interval (12 h), levels in serum should exceed 4.0 $\mu\text{g/ml}$. For that reason, an MIC breakpoint of ≤ 4.0 $\mu\text{g/ml}$ for the susceptible category seems to be reasonably conservative. In fact, all of our susceptible strains were inhibited by ≤ 2.0 $\mu\text{g/ml}$ in a microdilution test system.

TABLE 3. Summary of susceptibility tests with 15-, 30-, and 60- μg teicoplanin disks

Zone diam (mm)	No. of isolates in each MIC category ^a with disks containing (μg):								
	15			30			60		
	R	I	S	R	I	S	R	I	S
6	24			23			22		
7									
8									
9				1					
10									
11							1		
12									
13									
14			11			2			
15		1	17			17			
16			24			14			5
17			38		1	24	1		15
18			42			27			18
19			37			34		1	32
20			45			57			45
>20			46			85			145

^a R, Resistant (MIC, >8.0 $\mu\text{g/ml}$); I, intermediate susceptibility (MIC, 8.0 $\mu\text{g/ml}$); S, susceptible (MIC, ≤ 4.0 $\mu\text{g/ml}$). Susceptible breakpoints for tests with 15-, 30-, and 60- μg disks are ≥ 13 , ≥ 14 , and ≥ 15 mm, respectively.

For the standardized disk diffusion test, we recommend that 30- μg teicoplanin disks should be used, with breakpoints of ≤ 10 mm for resistant and ≥ 14 mm for susceptible strains. We also suggest that zone size breakpoints of ≤ 10 and ≥ 15 mm should be applied to tests with 30- μg vancomycin disks. This represents a minor change from the currently recommended standards of ≤ 9 and ≥ 12 mm for vancomycin.

Because of the marked bimodal distribution of endpoints, location of interpretive breakpoints is somewhat arbitrary, and regression statistics are not applicable. We prefer an intermediate range of at least 3 to 4 mm separating the susceptible and resistant categories to minimize the significance of minor technical variability that may influence the zone diameter. For that reason, we recommend changing the current standards for tests with 30- μg vancomycin disks. Similar zone size breakpoints can also be applied to tests with 30- μg teicoplanin disks, in spite of the fact that teicoplanin appears to diffuse much more slowly through the agar medium.

In the absence of gram-positive cocci that are truly resistant to either glycopeptide, routine susceptibility tests are not currently necessary. One could predict susceptibility to both drugs without doing any in vitro study, with a $>99\%$ predictive value. With the recommended interpretive breakpoints, both 30- μg disks performed with a $>99\%$ predictive value. One *Staphylococcus* sp. was moderately susceptible to teicoplanin but susceptible to vancomycin, and one strain of *Streptococcus faecium* was moderately susceptible to vancomycin but susceptible to teicoplanin. When retested, the MICs for both of those strains did not change. It is difficult to determine whether those two aberrant strains were truly resistant, but they were both susceptible by the disk tests. With the exception of those two aberrant strains, all vancomycin-susceptible strains were also susceptible to teicoplanin, and all teicoplanin-susceptible strains were also susceptible to vancomycin. Either disk could be used for predicting susceptibility to the other drug. However, since no resistant clinical isolates are

available at this time, we cannot determine whether resistance to one drug can be predicted from the results of tests with the other drug.

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