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Quality Control Limits for Teicoplanin Susceptibility Tests and Confirmation of Disk Diffusion Interpretive Criteria

ARTHUR L. BARRY,[1]* RONALD N. JONES,[1] THOMAS L. GAVAN,[2] CLYDE THORNSBERRY,[3] AND THE COLLABORATIVE ANTIMICROBIAL SUSCEPTIBILITY TESTING GROUP†

The Clinical Microbiology Institute, Tualatin, Oregon 97062[1]; The Cleveland Clinic Foundation, Cleveland, Ohio 44106[2]; and Centers for Disease Control, Atlanta, Georgia 30333[3]

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For monitoring the performance of teicoplanin susceptibility tests, the following quality control limits are recommended: *Staphylococcus aureus* ATCC 29213, MIC of 0.12 to 0.5 μg/ml; *Enterococcus faecalis* ATCC 29212, MIC of 0.06 to 0.25 μg/ml; and *S. aureus* ATCC 25923, zones 15 to 19 mm in diameter (30-μg disks). However, some lots of Mueller-Hinton agar provided unusually large zones of inhibition with both vancomycin and teicoplanin disks, and these lots should be excluded before routine use. Teicoplanin and vancomycin differed only in their activity against oxacillin-resistant strains of *Staphylococcus haemolyticus*, which had decreased susceptibility to teicoplanin but were fully susceptible to vancomycin. For tests with 30-μg teicoplanin disks, zones ≤10 and ≥14 mm in diameter represent resistant and susceptible breakpoints, respectively.

Teicoplanin is a complex of glycopeptide antibiotics which has a spectrum of antibacterial activity similar to that of vancomycin (1a, 2, 5, 8–10). Both drugs are primarily effective against gram-positive bacterial pathogens but not against gram-negative pathogens. The serum half-life of teicoplanin is markedly prolonged compared with that of vancomycin, but both drugs attain similar peak levels in blood (4, 10).

For disk diffusion susceptibility tests, we previously recommended the use of 30-μg teicoplanin disks, and tentative zone size interpretive criteria were proposed on the basis of studies performed in two separate laboratories with disks manufactured in one of the laboratories (1a). We now report the results of additional tests performed with commercially prepared disks (BBL Microbiology Systems, Cockeysville, Md.). In this confirmatory study, the coagulase-negative staphylococci were identified to the species level to better characterize any strains that were resistant to teicoplanin but susceptible to vancomycin.

Broth microdilution and disk diffusion susceptibility tests were performed in accordance with the procedures outlined by the National Committee for Clinical Laboratory Standards (6, 7). Two separate collaborative studies were performed to identify quality control parameters for disk diffusion and microdilution tests. The study design was that described by Gavan et al. (3) for disk tests or Barry et al. (1) for microdilution tests. In both studies, replicate tests were performed in at least five different laboratories, each using a different lot of Mueller-Hinton agar or a different lot of microdilution trays. For interlaboratory control, additional tests were performed with a single lot of agar plates or microdilution trays common to all testing facilities.

* Corresponding author.

† S. D. Allen, University of Indiana Medical Center, Indianapolis, IN 46202; L. W. Ayers, The Ohio State University Medical Center, Columbus, OH 43210; P. C. Fuchs, St. Vincent Hospital and Medical Center, Portland, OR 97225; E. H. Gerlach, St. Francis Hospital and Medical Center, Wichita, KS 67214; and L. B. Reller, University of Colorado Medical Center, Denver, CO 80220.

The results of replicate microdilution tests are shown in Table 1. The two gram-positive microorganisms that are normally used to monitor microdilution tests were evaluated in five different laboratories. Only one MIC varied by more than one doubling dilution above or below the mode for each strain. The proposed control limits for teicoplanin MICs are: *Staphylococcus aureus* ATCC 29213, 0.12 to 0.5 μg/ml, and *Enterococcus faecalis* ATCC 29212, 0.06 to 0.25 μg/ml.

The results of replicate disk susceptibility tests in six different laboratories are presented in Table 2. The control *S. aureus* strain was tested 330 times, and each time three different lots of teicoplanin disks and one lot of vancomycin disks were evaluated. Diameters of zones of inhibition ranged from 14 to 22 mm with teicoplanin disks and from 15 to 22 mm with vancomycin disks. For both disks, control limits of 15 to 21 mm would include 98% of all the data points for both drugs. However, the current National Committee for Clinical Laboratory Standards document (6) recommends control limits of 15 to 19 mm for tests with vancomycin disks. Two of the six participants reported all of the zones that were larger than the 19-mm upper limit. Both of these participants reported satisfactory results when they used the lot of Mueller-Hinton agar that was common to all testing facilities. Identical medium-dependent variations in teicoplanin zone sizes were also observed. If data from two

TABLE 1. Microdilution susceptibility tests

MIC (μg/ml)	No. of times MIC recorded[a]	
	S. aureus ATCC 29213	*E. faecalis* ATCC 29212
1.0	0	0
0.5	42	1
0.25	100	37
0.12	8	97
0.06	0	15
0.03	0	0

[a] Replicate determinations were made in five different laboratories (150 MICs for each control strain). The proposed upper and lower limits of acceptable variation in MICs are separated by horizontal lines.

TABLE 2. Disk susceptibility tests of *S. aureus* ATCC 25923

Zone diam (mm)	No. of times zone size recorded with 30-μg disks[a]			
	Vancomycin		Teicoplanin	
	All labs	Four labs[b]	All labs	Four labs[b]
13	0	0	0	0
14	0	0	18	18
15	22	22	99	99
16	36	36	105	105
17	59	59	233	233
18	99	91	215	196
19	22	12	104	9
20	45	0	145	0
21	42	0	67	0
22	5	0	4	0
23	0	0	0	0

[a] One lot of vancomycin disks and three lots of teicoplanin disks were tested 330 times, generating 990 teicoplanin zones and 330 vancomycin zones. Replicate determinations were made in six different laboratories. The currently recommended (6) zone size limits for tests with 30-μg vancomycin disks and the proposed limits for tests with 30-μg teicoplanin disks are indicated by horizontal lines.

[b] Two of the six laboratories tested media that produced unusually large zones of inhibition. The zones recorded by the other four laboratories are recorded separately.

of the six participants were excluded because their media were unsatisfactory, control limits of 15 to 19 mm could be applied to tests with teicoplanin disks as well as vancomycin disks.

We recommend continued use of 15 to 19-mm control limits for tests with vancomycin disks, and the same control limits should apply to tests with teicoplanin disks. However, our data suggest that some lots of Mueller-Hinton agar may produce unusually large zones of inhibition (20 to 22 mm), and each new lot of agar must be screened before use. Current efforts to better standardize the production of Mueller-Hinton agars are likely to improve this situation. It should be noted that a recent national survey by the College of American Pathologists has identified a specific problem with quality control of vancomycin disk tests. More than 10% of all recorded zone diameters were above the currently recommended maximum of 19 mm. By expanding the control limits to include zones of 15 to 21 mm, many of these problems would be resolved, but such a broad range of acceptable limits is not likely to provide useful quality control information.

To confirm the previously proposed tentative zone size breakpoints (1a), 338 bacterial isolates were tested against vancomycin and teicoplanin (Table 3). Although teicoplanin was generally more active than vancomycin, both drugs had similar spectra of activity. Oxacillin-resistant strains of *Staphylococcus haemolyticus* represent the only important exception to the rule of cross susceptibility. Among the 10 strains that we tested, 8 were resistant to oxacillin. The teicoplanin MICs for these eight strains were elevated, ranging from 2.0 to 32 μg/ml, but all of the strains were susceptible to vancomycin (MICs 1.0 to 4.0 μg/ml). The two oxacillin-susceptible strains were susceptible to teicoplanin (MICs, 0.12 and 0.5 μg/ml), as well as to vancomycin (MIC,

TABLE 3. Species used for evaluation of teicoplanin and vancomycin disk susceptibility tests

Species (no. of isolates tested)	MIC (range [μg/ml])		Zone size (range [mm])	
	Teicoplanin	Vancomycin	Teicoplanin	Vancomycin
Staphylococcus spp.				
S. aureus				
Methicillin susceptible (59)	0.12–0.5	0.5–1.0	18–21	19–22
Methicillin resistant (49)	0.06–1.0	0.25–2.0	16–20	16–21
S. epidermidis (10)	0.06–0.12	1.0–4.0	17–20	20–22
S. haemolyticus[a] (10)	0.12–32	1.0–4.0	14–20	19–22
S. saprophyticus (9)	0.06–1.0	0.5–2.0	17–20	19–20
Others[b] (10)	0.12–2.0	0.5–2.0	18–23	20–24
Streptococcus spp.				
S. pyogenes (19)	≤0.008–0.06	≤0.12–0.25	14–19	18–20
S. agalactiae (10)	0.06–0.06	0.25–0.5	16–17	18–21
Viridans group (11)	0.015–0.25	0.25–1.0	16–24	20–27
S. pneumoniae (20)	0.015–0.06	≤0.12–0.5	19–22	21–26
S. bovis (11)	0.06–0.25	0.25–0.5	20–21	23–25
Enterococcus spp.				
E. faecalis (10)	0.06–0.12	1.0–4.0	17–20	17–20
Others[c] (10)	0.06–0.5	0.5–4.0	16–18	14–21
Branhamella catarrhalis (19)	4.0–16	>16–>16	9–14	8–13
Corynebacterium JK group (10)	0.12–0.5	0.25–2.0	20–24	25–30
Gram-negative bacilli[d] (71)	>16–>16	>16–>16	6–6	6–11

[a] Includes two oxacillin-susceptible strains that were susceptible to teicoplanin (MICs, 0.12 and 0.5 μg/ml; zone sizes, 19 to 20 mm) and vancomycin (MIC, 1.0 μg/ml; zone size, 22 mm).

[b] Includes five *S. warneri* isolates, two *S. auricularis* isolates, two *S. simulans* isolates, and one *S. cohnii* isolate.

[c] Includes seven *E. faecium* isolates and three *E. durans* isolates.

[d] Includes the following isolates: 40 *Haemophilus influenzae* (20 beta-lactamase positive), 2 *Escherichia coli*, 1 *Citrobacter diversus*, 1 *Citrobacter freundii*, 1 *Enterobacter aerogenes*, 1 *Enterobacter agglomerans*, 1 *Enterobacter cloacae*, 2 *Klebsiella pneumoniae*, 2 *Serratia marcescens*, 2 *Proteus mirabilis*, 2 *Proteus vulgaris*, 2 *Morganella morganii*, 2 *Providencia rettgeri*, 2 *Providencia stuartii*, 2 *Acinetobacter calcoaceticus* subsp. *anitratus*, 2 *Pseudomonas aeruginosa*, 2 *Pseudomonas cepacia*, 2 *Pseudomonas maltophilia*, and 2 *Pseudomonas stutzeri*.

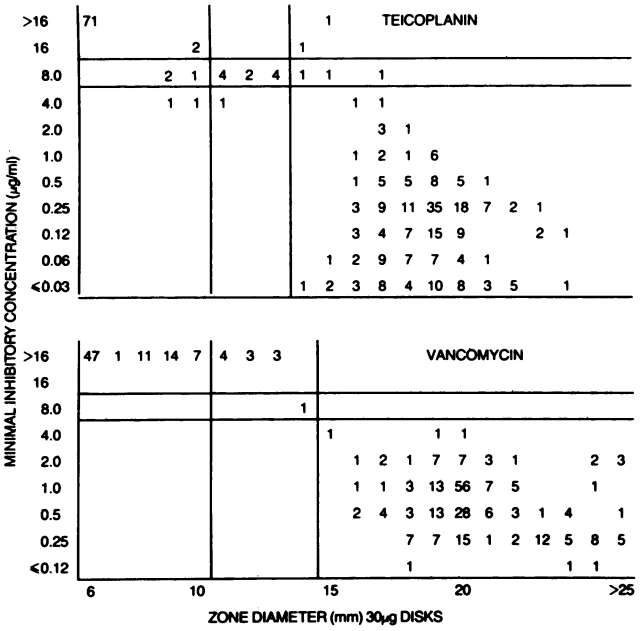

FIG. 1. Scattergrams correlating results of disk susceptibility tests with those of broth microdilution susceptibility tests. A total of 338 strains were tested (Table 3). The MIC and zone size breakpoints (horizontal and vertical lines) are those previously proposed (1a).

1.0 μg/ml). *Branhamella catarrhalis* strains also differed in their response to the two glycopeptides. Although these gram-negative microorganisms would normally be considered resistant to both drugs, 14 of 19 strains were intermediate in teicoplanin resistance (MIC, 8.0 μg/ml), 3 were susceptible (MIC, 4.0 μg/ml), and 1 was resistant (MIC, 16 μg/ml). However, all 19 strains were resistant to vancomycin (MICs, >16 μg/ml).

The correlation between zone sizes and vancomycin MIC are shown in Fig. 1. Interpretive criteria that we have previously recommended (1a) are superimposed on the scattergrams. With 30-μg vancomycin disks, the zones for the *B. catarrhalis* isolates ranged from 8 to 13 mm in diameter, although the MICs were all >16 μg/ml. For one strain of *Enterococcus faecium*, the MIC was 8.0 μg/ml (intermediate); this strain had an intermediate-size zone of 14 mm. The previously recommended zone size criteria of ≤10 and ≥15 mm appear to be quite appropriate for tests with 30-μg vancomycin disks.

A scattergram for teicoplanin test results is also shown in Fig. 1. With 30-μg teicoplanin disks, the zones for 19 *B. catarrhalis* strains were 9 to 14 mm in diameter; 11 of the 19 strains had intermediate zone sizes (11 to 13 mm), 1 was susceptible (14 mm), and 7 were resistant (9 or 10 mm). The last group included two strains that were susceptible by the MIC test. The teicoplanin MICs for the eight oxacillin-resistant *S. haemolyticus* isolates were elevated, but the isolates were all susceptible by the disk test (≥14-mm zones). Only two of the eight isolates provided very major errors (resistant by the MIC test but susceptible by the disk test); two others were intermediate by the MIC test but susceptible by the disk test. The four remaining isolates were susceptible by both methods. Complete interpretive agreement was achieved in 96.7% of all teicoplanin tests; this is comparable to the 97.0% interpretive agreement in the vancomycin tests. We concluded that the proposed interpretive breakpoints perform satisfactorily for both 30-μg teicoplanin and 30-μg vancomycin disks, pending correlation with the outcome of clinical trials.

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