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Ciprofloxacin Disk Susceptibility Tests: Interpretive Zone Size Standards for 5-μg Disks

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Evaluations of 5-μg ciprofloxacin disk diffusion susceptibility tests were performed independently by seven different investigators. The results of the separate tests were combined to increase the number of resistant strains in the challenge set of microorganisms. Based on data with 2,652 isolates, the following interpretive breakpoints are tentatively proposed for use in ongoing clinical trials of ciprofloxacin: ≤15 mm, resistant (MIC > 2.0 μg/ml); 16 to 20 mm, intermediate (1.0 < MIC ≤ 2.0 μg/ml); and ≥21 mm, susceptible (MIC ≤ 1.0 μg/ml). Disk tests with *Streptococcus* spp. and with *Pseudomonas maltophilia* were not reliable; other microorganisms were accurately categorized by the disk diffusion test.

Ciprofloxacin is a quinolone antimicrobial agent which is being developed for therapy of systemic as well as urinary tract infections. It may be administered orally or parenterally. By either route, levels in serum in excess of 1.0 μg/ml are readily achieved (6–8, 11, 12, 20). Because the vast majority of bacterial pathogens are inhibited by less than 0.1 μg of ciprofloxacin per ml (2, 4, 5, 9, 10, 13, 15, 18, 19), it should be an effective broad-spectrum therapeutic agent.

As part of early in vitro evaluations, the disk susceptibility test of Bauer et al. (3) was evaluated in six different independent laboratories. The sparsity of resistant strains made it difficult to reach any uniform recommendations concerning interpretive zone-size standards. The independent investigators conferred and concluded that the six sets of data should be combined and that additional data with ciprofloxacin-resistant clinical isolates should be added by a seventh investigator. In the present report, we describe the results of such a combined effort and make specific recommendations for the interpretation of tests with 5-μg ciprofloxacin disks.

MATERIALS AND METHODS

Antimicrobial susceptibility tests. Disk diffusion tests were performed by the procedure of Bauer et al. (3) as modified by the National Committee for Clinical Laboratory Standards (17). Two investigators used 5-μg ciprofloxacin disks that were prepared at The Clinical Microbiology Institute, Tualatin, Oreg., and later confirmed by independent assay as having satisfactory potency. All other investigators used a single lot of 5-μg ciprofloxacin disks prepared by BBL Microbiology Systems, Cockeysville, Md.

Broth microdilution tests were performed by the procedure of the National Committee for Clinical Laboratory Standards (16) in five of the participating laboratories. Two other investigators used an agar dilution technique, as outlined by the National Committee for Clinical Laboratory Standards (16). One of those investigators used a dilution scheme that involved serial dilutions starting at 25 μg/ml. All others used twofold dilutions that provided concentrations on an even $\log_2$ scale (starting at 32 μg/ml). All investigators tested concentrations of 0.008 μg/ml or less. MICs ≤ 0.008 μg/ml were recorded as 0.008 μg/ml unless lower concentrations were tested. Since regression analysis was not applicable, there was no need to exclude off-scale MIC values.

Quality control strains included *Streptococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 (for disk tests) and ATCC 29213 (for dilution tests). At least 60 MICs or zone diameters were recorded for each control strain.

RESULTS

Data accumulated with the standard control strains were first examined to evaluate the comparability of methods. Modal MICs were as follows: *E. coli* ATCC 25922, ≤0.008 μg/ml; *P. aeruginosa* ATCC 27853, 0.5 μg/ml; *Streptococcus faecalis* ATCC 29212, 1.0 μg/ml; and *S. aureus* ATCC 29213, 0.25 μg/ml. With the *E. coli* control strain, 60% of the reported MICs were ≤0.015 μg/ml. One of the investigators reported MICs of 0.03 or 0.06 μg/ml, which skewed the data over a range of four dilution intervals. Later investigations failed to reveal an explanation for the discrepant results from that laboratory; other drugs tested at the same time with the *E. coli* strain gave results that were within accepted control limits. With the *P. aeruginosa* control strain, 92% of all reported MICs were within a range representing the mode ± one doubling dilution. With the two gram-positive control strains, all reported MICs were within one dilution interval of the mode. Control data accumulated with the disk diffusion test also revealed reasonable comparability between participating laboratories. Consequently, data from all seven investigators were combined for analysis.

The combined data are presented in Fig. 1 and 2. Tests with the 2,652 bacterial isolates included 2,394 susceptible isolates (MICs ≤ 1.0 μg/ml), 174 isolates which were intermediate in susceptibility (1.0 < MIC ≤ 2.0 μg/ml), and 83 resistant isolates (MIC > 2.0 μg/ml). Streptococci made up

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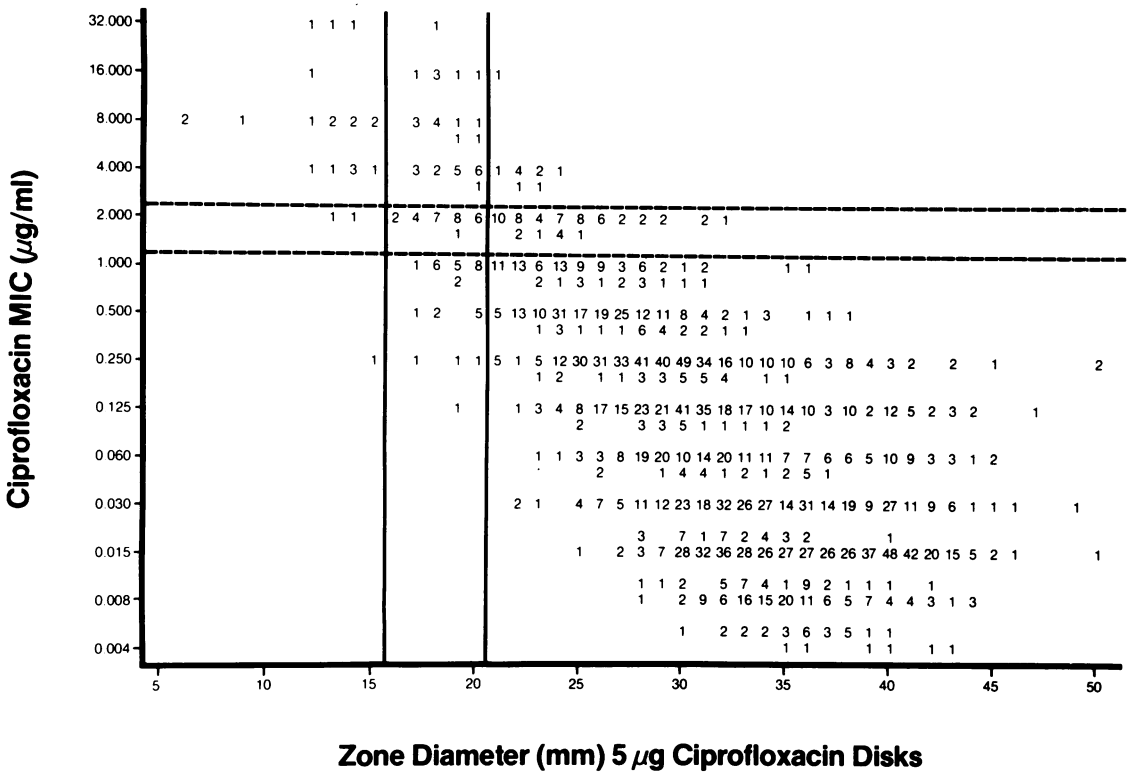

FIG. 1. Evaluation of 5-μg ciprofloxacin disks for testing the susceptibility of 1,959 gram-negative bacilli and 360 staphylococci. Data were accumulated from seven independent laboratories.

84 of the 174 strains with intermediate MICs and 17 of the 83 resistant strains.

The error rate-bounding technique of Metzler and DeHaan (14) was used to analyze the data with gram-negative bacilli and staphylococci. That analytical method requires the selection of a single MIC breakpoint to separate susceptible and resistant strains. To accomodate the intermediate MIC category, two analyses were applied, one involving the use of an MIC of ≤1.0 μg/ml for the susceptible category and the other involving the use of a breakpoint of ≤2.0 μg/ml for the susceptible category. By combining the two analyses, three MIC categories were accomodated. Because of the large proportion of strains that were very susceptible to ciprofloxacin, the percentage with interpretive errors was relatively small. Therefore, the 1 and 5% acceptable error rates that were proposed by Metzler and DeHaan (14) are not appropriate. The interpretive errors that would be generated by several alternative zone size breakpoints are listed in Table 1. Breakpoints of ≤15 mm and ≥21 mm for resistant and susceptible strains, respectively, were selected after examining the scattergram (Fig. 1). Only 4.4% of the tests with microorganisms other than streptococci had zones in the intermediate range of 16 to 20 mm. Major and very major error rates were 0.04 and 0.5%, respectively, with MIC breakpoints of ≤ 1.0 μg/ml and > 2.0 μg/ml for susceptible and resistant strains, respectively. Of the 333 streptococci, 54.9% produced zones in the intermediate category, and the error rates were 6.0% (major) and 0.3% (very major) (Fig. 2).

Intermediate zones of 16 to 20 mm were seen with 286 (10.8%) isolates, including 183 *Streptococcus* spp., 118 of which were susceptible by MIC methods and 12 of which were resistant. Intermediate-sized zones were obtained with 20 of 61 *P. maltophilia* isolates; 16 of those 20 strains were resistant by MIC methods. With other microorganisms, intermediate zone sizes were relatively uncommon.

With zone size standards of ≤15 mm of resistant and ≥21 mm for susceptible strains, the disk test agreed with the MIC categories with 87.7% of all isolates: 11% of the nonagreements were considered minor discrepancies. Table 2 describes the discrepancies between disk tests and MIC categories by organism group. Nearly half the streptococci and over half the *P. maltophilia* isolates showed minor discrepancies between the two types of tests. False-resistant disk tests (major errors) were recorded with 21 (0.8%) strains: 20 of the 21 major errors involved tests with streptococci, and the other involved a susceptible *Staphylococcus* sp. with a zone of 15 mm. Among the 2,652 isolates tested, there were 12 false-susceptible disk test results (very major errors): 6 of the 12 very major errors occurred with *P. maltophilia* strains. The disk test was highly reliable (>97% agreement) when testing the *Enterobacteriaceae* family and *Staphylococcus* spp., but there were no resistant staphylococci and only seven resistant enteric bacilli in the collection. Intermediate disk test results were seen with four of the seven resistant enteric bacilli: one was susceptible and two were resistant by the disk test.

Of the 22 gram-negative bacilli that were resistant by the disk test, 20 were also resistant by MIC methods (90.9% predictive value). The disk test reported 44 streptococci to be resistant, but only 4 of those strains were confirmed to be resistant by MIC methods (9.1% predictive value). Disk diffusion tests with streptococci and with *P. maltophilia* provided little useful information. Of 61 *P. maltophilia* isolates, 30 were resistant by MIC methods but only 8 were resistant by the disk test: 33 strains appeared to be susceptible by the disk test, but only 12 of those were susceptible

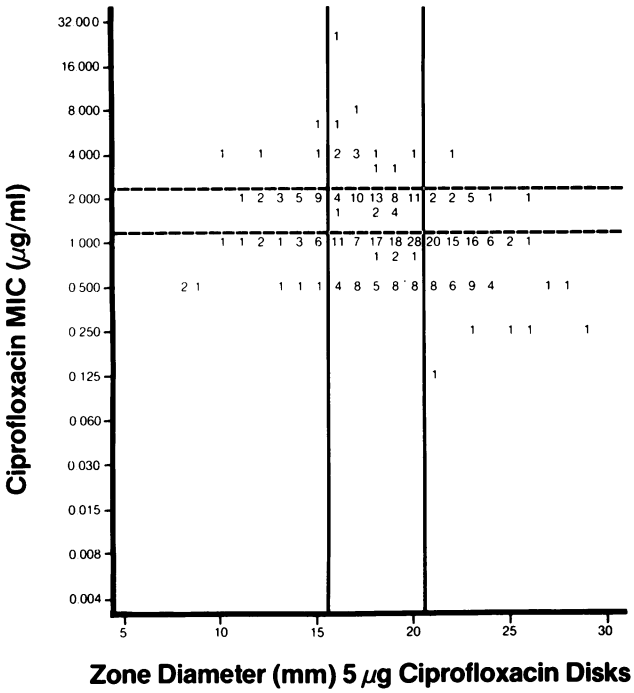

FIG. 2. Evaluation of 5-μg ciprofloxacin disks for testing the susceptibility of 333 streptococci (150 enterococci, 78 *Streptococcus agalactiae*, 34 *Streptococcus pyogenes*, 34 *Streptococcus pneumoniae*, and 37 viridans group). Data were accumulated from seven independent laboratories.

by MIC methods (36.4% predictive value). An intermediate disk test result had no useful predictive value and should be interpreted as indicating an equivocal or indeterminate test result rather than indicating an intermediate degree of susceptibility. Among the 1,387 members of the family *Enterobacteriaceae*, 1,355 isolates were susceptible, 25 were intermediate, and 7 were resistant by MIC methods. If one assumed all enteric bacilli to be susceptible, without any testing, the predictive value would be 97.7% (1,355/1,387). On the other hand, when a disk test was performed, 30 (2.2%) provided indeterminate results, and a resistant disk test result was obtained with 4 strains (2 of those were resistant by MIC methods and 2 were intermediate). Of the 1,352 isolates with a susceptible disk test result, 1,340 were susceptible, 11 were intermediate, and one was resistant (99.1% predictive value). This result can be compared with the 97.7% predictive value if no test is done.

DISCUSSION

The foregoing evaluation was based on the assumption that microorganisms that are inhibited by 1.0 μg of ciprofloxacin per ml should be responsive to oral or intravenous therapy. Crump et al. (7) documented peak levels in serum of 2.4 μg/ml after oral administration of a 500-mg tablet. The half-life in serum was 3.9 h, and therefore levels in serum of >1.0 μg/ml should be maintained for 4 to 5 h after administration. In the same subjects, peak concentrations of ciprofloxacin in blister fluid were approximately 1.0 μg/ml at 3 to 4 h after administration. Gonzalez et al. (11) documented increased levels in blood as a result of oral doses of 250, 500, and 750 mg. After intravenous administration of a 100-mg bolus injection in healthy volunteers, concentrations of approximately 0.5 μg/ml were found in serum and blister fluid (20). Ciprofloxacin, like other quinolone derivatives, is capable of selecting resistant populations when microorganisms are exposed in vitro to subinhibitory concentrations (1). For obvious reasons, prolonged exposure to subinhibitory concentrations of ciprofloxacin at the site of infection should be avoided. Consequently, strains for which MICs are ≤1.0 μg/ml are considered susceptible to ciprofloxacin and those for which MICs are >2.0 μg/ml are considered resistant. Most resistant strains may be responsive to ciprofloxacin if a lower-urinary-tract infection is being treated. These MIC breakpoints should be considered tentative guidelines for use during ongoing clinical trials. When more clinical experience has been gained with ciprofloxacin, the MIC breakpoints should be reevaluated and disk test standards should be adjusted accordingly.

The current report describes data obtained only with 5-μg ciprofloxacin disks. Most investigators studied other disk potencies, but they all agreed initially to select the 5-μg disk because 10-μg disks provided no advantages. Less potent disks have not yet been studied as extensively as the 5-μg disk.

Even with the large number of strains tested in the current study, the number of resistant isolates is very small. Consequently, it is difficult to define zone size interpretive standards with a great deal of confidence. Significant interpretive errors were minimal because of a rather broad (16- to 20-mm) intermediate category. Most of the microorganisms with intermediate-sized zones were *P. maltophilia* or streptococci. Only 3.7% of the isolates other than *P. maltophilia*

TABLE 1. Interpretive errors generated by applying selected zone size breakpoints for interpretation of 5-μg ciprofloxacin disk tests with 2,319 isolates[a]

Zone diam breakpoints (mm)			% Total agreement	No. (%) of discrepant tests			No. (%) with intermediate zones
R[b]	I[b]	S[b]		False-resistant[c]	False-susceptible[c]	Minor errors[d]	
≤13	14–18	≥19	93.7	0	29 (1.2)	117 (5.0)	57 (2.5)
≤14	15–19	≥20	93.9	0	21 (0.9)	120 (5.2)	76 (3.3)
≤14	15–20	≥21	93.5	0	11 (0.5)	139 (6.0)	107 (4.6)
≤15	16–19	≥20	94.0	1 (0.04)	21 (0.9)	116 (5.0)	72 (3.1)
≤15	16–20	≥21	93.7	1 (0.04)	11 (0.5)	135 (5.8)	103 (4.4)
≤16	17–20	≥21	93.6	1 (0.04)	11 (0.5)	137 (5.9)	101 (4.4)
≤16	17–21	≥22	93.1	1 (0.04)	9 (0.4)	150 (6.5)	134 (5.8)

[a] This analysis excludes data obtained with 333 streptococci (see Fig. 2).
[b] R, Resistant; I, intermediate; S, susceptible.
[c] Based on MIC categories of ≤1.0 μg/ml for susceptible, >2.0 μg/ml for resistant, and >1.0 but ≤2.0 μg/ml for intermediate strains.
[d] Intermediate with one method but susceptible or resistant with the other method.

TABLE 2. Microorganisms with interpretive discrepancies between ciprofloxacin disk tests and MIC categories[a]

Microorganism (no. tested)	No. (%) of discrepant[b] results			% Agreement
	Minor	Major	Very major	
Enterobacteriaceae (1,387)	32 (2.3)	0	1 (0.07)	97.6
Acinetobacter (113)	14 (12.4)	0	0	87.6
P. aeruginosa (275)	30 (10.9)	0	2 (0.7)	88.4
P. maltophilia (61)	32 (52.4)	0	6 (9.8)	37.7
Other *Pseudomonas* spp. (66)	10 (15.2)	0	2 (3.0)	81.8
Other gram-negative bacilli (57)	5 (8.8)	0	0	91.2
Staphylococcus spp. (360)	8 (2.2)	1 (0.3)	0	97.5
Streptococcus spp. (333)	161 (48.3)	20 (6.0)	1 (0.3)	45.3
All organisms (2652)	292 (11.0)	21 (0.8)	12 (0.4)	87.7

[a] Susceptible, ≥21 mm (MIC ≤ 1.0 μg/ml); intermediate, 16 to 20 mm (1.0 < MIC ≤ 2.0 μg/ml); resistant, ≤15 mm (MIC > 2.0 μg/ml).
[b] Minor, intermediate with one method, susceptible or resistant with the other; major, susceptible by MIC but resistant by the disk test; very major, resistant by MIC but susceptible by the disk test.

or streptococci gave intermediate disk test results. Most of the minor discrepancies between disk tests and MIC categories involved tests with streptococci and with *P. maltophilia*. Separate interpretive criteria for these two types of microorganisms would not have appreciably improved the reliability of the disk test. With other microorganisms, the disk test was reasonably reliable. An intermediate disk test result is considered an indeterminate or equivocal result. A susceptible disk test result had a >99% predictive value when staphylococci or enteric bacilli were tested. For all organisms other than streptococci and *P. maltophilia*, a resistant disk test result had a 98% predictive value. If streptococci or *P. maltophilia* isolates are to be tested, the disk diffusion procedure should not be used; a dilution test is preferred.

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