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Proposed Interpretive Criteria and Quality Control Parameters for Testing In Vitro Susceptibility of *Neisseria gonorrhoeae* to Ciprofloxacin

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Ciprofloxacin was subjected to a multilaboratory study designed to determine its in vitro susceptibility criteria for *Neisseria gonorrhoeae* and its quality control parameters for both agar dilution and disk diffusion susceptibility testing for this species. All clinical isolates were susceptible, i.e., MICs were ≤0.06 μg/ml and zones of inhibition were ≥36 mm. A resistant category could not be defined, but in vitro-selected mutants gave zones of ≤35 mm, and MICs for these strains were ≥0.12 μg/ml. For quality control of ciprofloxacin agar dilution tests on supplemented GC agar, MICs for *Staphylococcus aureus* ATCC 29213 ranged from 0.12 to 0.5 μg/ml. For quality control of 5-μg ciprofloxacin disk tests, *N. gonorrhoeae* ATCC 49226 and *S. aureus* ATCC 25923 produced acceptable zone diameter ranges of 48 to 58 mm and 22 to 26 mm, respectively.

Ciprofloxacin has been demonstrated to be effective in the treatment of gonococcal infections, including those caused by penicillinase-producing *Neisseria gonorrhoeae* (2, 7, 11, 12). The Centers for Disease Control has listed ciprofloxacin among the alternative drugs for therapy of gonorrhea (3).

The interpretive criteria and quality control parameters for ciprofloxacin susceptibility testing in vitro are well established for nonfastidious rapidly growing aerobic and facultatively anaerobic bacteria (9, 10). Such criteria and parameters are not yet established for *N. gonorrhoeae*. The purposes of the present study were (i) to develop interpretive criteria for ciprofloxacin susceptibility testing of *N. gonorrhoeae* by agar dilution and disk diffusion methods and (ii) to develop quality control parameters for both methods by multilaboratory testing in accordance with the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (8).

MATERIALS AND METHODS

Ciprofloxacin was provided as a standardized powder by Miles, Inc., West Haven, Conn. Susceptibility testing was performed in accordance with the methods recommended by the NCCLS for agar dilution and disk diffusion testing of *N. gonorrhoeae* (9, 10). GC agar supplemented with 1% defined XV devoid of cysteine was used in all tests. Each of 102 *N. gonorrhoeae* isolates was tested in triplicate by both methods, and the modal MIC for each isolate was plotted against the mean inhibitory zone diameter. The 102 isolates included 21 penicillinase-producing *N. gonorrhoeae* strains; 28 penicillinase-negative, penicillin-resistant *N. gonorrhoeae* strains; and 53 penicillinase-negative, penicillin-susceptible *N. gonorrhoeae* strains. Because none of the 102 isolates was resistant to ciprofloxacin, 22 resistant strains were induced by repeatedly exposing the isolates to increasing concentrations of various fluoroquinolones. The method was as previously described (1) except that a heavier suspension (equivalent to a 1.0 McFarland standard) was inoculated onto GC agar plates with appropriate supplementation instead of in broth. The selective agents were ciprofloxacin, enoxacin, and ofloxacin, all of which induced resistance to all four compounds over 10 passages. The results of tests with these 22 strains with induced resistance are included in the plots as strains for which MICs were ≥0.12 μg/ml. The resistant mutants that were selected for this purpose have remained resistant after 5 months of storage, although the tests were performed immediately after the serial passages were completed.

For development of the quality control parameters, tests were conducted in the laboratories directed or supervised by each of the five authors. Antibiotic powders of known potency were provided to each facility together with a supplement and two lots of GC agar (one lot common to all participants and the other lot unique to that facility). The six lots of GC agar were obtained from three different manufacturers: BBL Microbiology Systems (Cockeysville, Md.) (lot nos. A2DVHH, I1GURL, and B1DTFZ), Difco (Detroit, Mich.) (lot nos. 774904 and 770612), and Oxoid USA, Inc. (Columbia, Md.) (lot no. R/42167). For the agar dilution test, the agar plates were prepared with twofold dilutions of ciprofloxacin on the day of testing. The test procedure was that outlined by the NCCLS (10). Replicate tests were performed with *N. gonorrhoeae* ATCC 49226 and *Staphylococcus aureus* ATCC 29213 until 20 MICs for each strain had been generated by each laboratory with the laboratory's unique lot and 5 MICs for each strain had been produced with the common lot of GC agar. Each replicate test represented a different inoculum preparation.

For the disk diffusion quality control tests, three different lots of 5-μg ciprofloxacin disks were evaluated in addition to the six lots of GC agar. The disk diffusion test recommended by the NCCLS was used throughout (7). Each laboratory performed 50 separate tests with each control strain (*N. gonorrhoeae* ATCC 49226 and *S. aureus* ATCC 25923). Each test involved three different lots of 5-μg ciprofloxacin disks (BBL 806589, Difco 691347, and G-D 110205 [Organon

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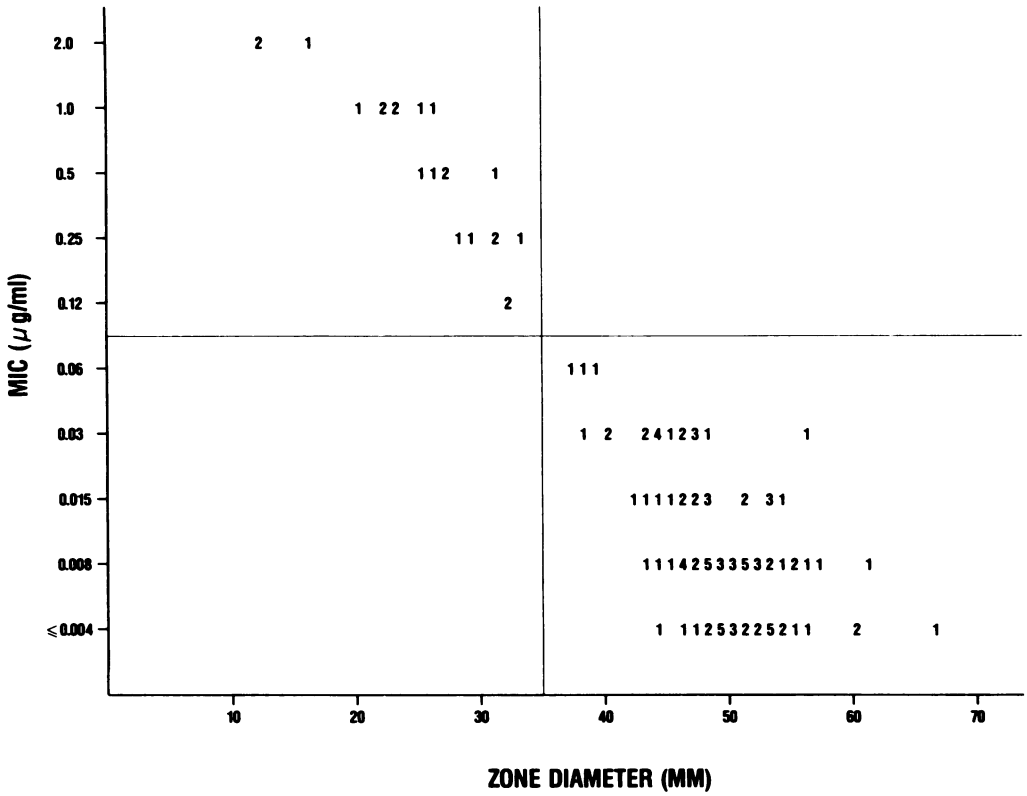

FIG. 1. Scattergram of ciprofloxacin MICs (mode of three tests) and mean zone diameters around 5-μg ciprofloxacin disks for 124 *N. gonorrhoeae* isolates. The 22 strains for which MICs were ≥0.12 μg/ml were all strains with laboratory-induced resistance, and the 102 clinical isolates were all inhibited by ≤0.06 μg/ml.

Teknika Corp., Durham, N.C.]) and a single 30-μg tetracycline disk for control purposes. In addition to performing 50 tests with its unique GC agar lot, each laboratory also performed 5 tests with the GC agar lot common to all facilities. Thus, each laboratory generated 150 zone diameter measurements with 5-μg ciprofloxacin disks using its unique lot of GC agar and 15 measurements with the common lot of GC agar.

RESULTS

The ciprofloxacin MICs for the 102 clinical *N. gonorrhoeae* isolates were all ≤0.06 μg/ml (susceptible). Penicillinase production appeared to have no effect on ciprofloxacin MICs, since median and modal ciprofloxacin MICs were 0.008 μg/ml for both penicillinase-producing and penicillinase-negative, penicillin-susceptible *N. gonorrhoeae* strains. Penicillinase-negative, penicillin-resistant strains, however, were somewhat less susceptible to ciprofloxacin; the ciprofloxacin modal and median MICs for that group were both 0.015 μg/ml. All isolates for which ciprofloxacin MICs were 0.06 μg/ml were penicillinase-negative, penicillin-resistant strains. For the 22 isolates with in vitro-selected resistance, ciprofloxacin MICs ranged from 0.125 to ≥2.0 μg/ml. All 102 susceptible clinical isolates had zone diameters of inhibition around the 5-μg ciprofloxacin disks of ≥36 mm; by contrast, all 22 laboratory-induced resistant strains had zone diameters of ≤35 mm (Fig. 1).

The results of replicate ciprofloxacin agar dilution testing of the two quality control organisms are summarized in Table 1. The ciprofloxacin MICs generated for *N. gonorrhoeae* ATCC 49226 ranged from ≤0.0005 to 0.008 μg/ml, a 5-dilution range. On the other hand, the ciprofloxacin MICs for *S. aureus* ATCC 29213 ranged from 0.12 to 0.5 μg/ml, a 3-dilution range that includes concentrations that are likely to be tested in most laboratories.

The data from replicate ciprofloxacin disk diffusion susceptibility testing with two quality control strains are summarized in Table 2. No appreciable difference in zone diameters was observed among the three different lots of 5-μg ciprofloxacin disks tested against either organism. The mean (standard deviation) zone diameters (in millimeters) for the three disk lots tested against *N. gonorrhoeae* ATCC 49226 were 52.7 (2.09), 53.1 (2.35), and 53.0 (2.45) for BBL 806539, Difco 766960, and G-D 110295, respectively. The corresponding values for the three disk lots tested against *S. aureus* ATCC 25923 were 23.8 (1.08), 24.0 (1.02), and 24.2 (1.15), respectively. Each of the quality control organisms was tested 275 times with 30-μg tetracycline disks; 98% of the zone diameters for *N. gonorrhoeae* ATCC 49226 fell within the recommended acceptable range of 30 to 42 mm (9), and 95% of zone diameters for *S. aureus* ATCC 25923 fell within the recommended acceptable range of 27 to 33 mm (9).

DISCUSSION

One difficulty in determining the ciprofloxacin MIC breakpoints for distinguishing susceptible and resistant *N. gonorrhoeae* strains is the current paucity of ciprofloxacin-resis-

TABLE 1. Ciprofloxacin MICs reported by five laboratories, each using a different lot of GC agar plus a sixth control lot common to all laboratories

Organism and laboratory	No. of times each of the following MICs (μg/ml) was reported[a]							
	≤0.005	0.001	0.002	0.004	0.008	0.012	0.25	0.5
N. gonorrhoeae ATCC 49226								
A		20 (5)						
B			7 (1)	13 (4)				
C					20 (5)			
D		20 (0)	0 (5)					
E	20 (5)							
Total	20 (5)	40 (5)	7 (6)	13 (4)	20 (5)			
S. aureus ATCC 29213								
A							20 (5)	
B							9 (5)	11 (0)
C								20 (5)
D							3 (0)	17 (5)
E						20 (5)		
Total						20 (5)	32 (10)	48 (10)

[a] Number of times each MIC was reported by each laboratory with a common lot is noted in parentheses; all other data obtained with lots unique to each laboratory.

tant strains. Three recent publications reported cure rates of 98% (48 of 49 patients), 99% (118 of 119 patients), and 100% (all 164 patients) of uncomplicated gonococcal infections with single-dose ciprofloxacin therapy (2, 11, 12). In these studies, the *N. gonorrhoeae* isolates were all inhibited by ≤0.03 μg of ciprofloxacin per ml in vitro. Strains that are more resistant to ciprofloxacin have been described recently (5, 6), but the clinical efficacy of ciprofloxacin in treating infections due to such strains is not yet known. Because ciprofloxacin-resistant clinical isolates were not available, ciprofloxacin resistance was induced in vitro, and resistant strains were then tested by both disk diffusion and agar dilution methods. Agar dilution data are included in Fig. 1: the laboratory-induced resistant strains can be distinguished from the susceptible clinical isolates since for the clinical isolates, MICs were ≤0.06 μg/ml and zone diameters were ≥36 mm. Until the clinical and bacteriologic response rates for such microorganisms are documented, we believe that it is prudent to simply define a susceptible category which includes the commonly encountered clinical isolates that are known to be responsive. Strains that fail to fall within that susceptible category (MICs of ≤0.06 μg/ml or zone diameters of ≥36 mm) are not necessarily resistant, but until data correlating clinical responses with higher MICs are obtained, such strains cannot be considered susceptible.

The ciprofloxacin agar dilution MICs for *N. gonorrhoeae* ATCC 49226 generated in this study were extremely low, ranging from ≤0.0005 to 0.008 μg/ml. This 5-dilution range appears to be laboratory dependent rather than medium dependent. Each laboratory demonstrated good intralabora-

TABLE 2. Mean and range of zone diameters obtained with 5-μg ciprofloxacin disks and two quality control (QC) strains

Organism and laboratory	Unique lot		Common lot		Proposed QC Range[a]	% in range
	No. of tests	Mean zone diameter (range)[b]	No. of tests	Mean zone diameter (range)[b]		
N. gonorrhoeae ATCC 49226					48–58	
A	150	52.3 (48–56)	15	50.8 (48–54)		100
B	150	51.3 (49–54)	15	49.9 (49–51)		100
C	150	52.4 (48–60)	15	48.7 (47–50)		98.8
D	150	54.9 (50–60)	15	52.2 (50–54)		98.8
E	150	53.9 (49–60)	15	50.8 (46–55)		95.8
Combined	750	53.0 (48–60)	75	50.5 (46–55)		98.7
S. aureus ATCC 25923					22–26	
A	150	23.1 (20–26)	15	24.5 (23–25)		89.7
B	150	24.1 (23–25)	15	23.9 (23–24)		100
C	150	24.2 (23–26)	15	22.9 (22–24)		100
D	150	24.2 (22–26)	15	24.3 (22–25)		100
E	150	24.5 (22–26)	15	23.5 (23–25)		100
Combined	750	24.0 (20–26)	75	23.8 (22–25)		98.0

[a] Derived from the method of Gavan et al. (4).
[b] In millimeters.

tory reproducibility (1 to 2 dilutions) with no significant difference in results obtained from unique and common lots of GC agar. Because of the wide MIC range reported herein and because the ciprofloxacin MIC for *N. gonorrhoeae* ATCC 49226 is well below the lowest concentrations that would reasonably be tested in clinical laboratories, we recommend that this organism not be tested with ciprofloxacin routinely for quality control purposes. Tests with *S. aureus* ATCC 29213, however, yielded ciprofloxacin MICs of 0.12 to 0.5 μg/ml. This 3-dilution range is well within the range of routine susceptibility testing of this drug, and therefore, testing with this organism is recommended for quality control purposes. This is the same range that is currently recommended by the NCCLS for control of dilution tests using Mueller-Hinton broth (10).

With 5-μg ciprofloxacin disks, the mean zone diameter of inhibition was 53.0 mm when *N. gonorrhoeae* ATCC 49226 was repeatedly tested in five laboratories. The standard deviation for all laboratories was 2.31 mm. By the method of Gavan et al. (4), the calculated acceptable range for this strain is 48 to 58 mm. With this as a proposed acceptable quality control range, 99% of the test results in this study would be considered acceptable. The mean zone diameter (standard deviation) for *S. aureus* ATCC 25923 was 24.0 (1.07) mm. By the same calculation procedure, the proposed acceptable quality control range for this strain is 22 to 26 mm. With this range, 98% of the test results in this study would be considered acceptable.

In summary, based on these studies, the following recommendations are tentatively proposed. (i) For ciprofloxacin, susceptible strains are those for which MICs are ≤0.06 μg/ml or zone diameters are ≥36 mm. No resistant category is defined. (ii) For quality control of ciprofloxacin agar dilution tests with supplemented GC agar, MICs for *S. aureus* ATCC 29213 may range from 0.12 to 0.5 μg/ml. (iii) For quality control of disk diffusion tests on supplemented GC agar, acceptable zone diameter limits for *N. gonorrhoeae* ATCC 49226 are 48 to 58 mm, and limits for *S. aureus* ATCC 25923 are 22 to 26 mm.

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REFERENCES

1. **Barry, A. L., and R. N. Jones.** 1984. Cross-resistance among cinoxacin, ciprofloxacin, DJ-6783, enoxacin, nalidixic acid, norfloxacin, and oxolinic acid after in vitro selection of resistant populations. Antimicrob. Agents Chemother. **25**:775–777.
2. **Bryan, J. P., S. K. Hira, W. Brady, N. Luo, C. Mwale, G. Mpoko, R. Krieg, E. Siwiwaliondo, C. Reicart, C. Waters, and P. L. Perine.** 1990. Oral ciprofloxacin versus ceftriaxone for the treatment of urethritis from resistant *Neisseria gonorrhoeae* in Zambia. Antimicrob. Agents Chemother. **34**:819–822.
3. **Centers for Disease Control.** 1989. 1989 sexually transmitted diseases treatment guidelines. Morbid. Mortal. Weekly Rep. **38**(S-8):1–43.
4. **Gavan, T. L., R. N. Jones, A. L. Barry, P. C. Fuchs, E. H. Gerlach, J. M. Matsen, L. B. Reller, C. Thornsberry, and L. D. Thrupp.** 1981. Quality control limits for ampicillin, carbenicillin, mezlocillin, and piperacillin disk diffusion susceptibility tests: a collaborative study. J. Clin. Microbiol. **14**:67–72.
5. **Gransden, W. R., C. A. Warren, I. Phillips, M. Hodges, and D. Barlow.** 1990. Decreased susceptibility of *Neisseria gonorrhoeae* to ciprofloxacin. Lancet **335**:51.
6. **Jephcott, A. E., and A. Turner.** 1990. Ciprofloxacin resistance in gonococci. Lancet **335**:165.
7. **Moran, J. S., and J. M. Zenilman.** 1990. Therapy for gonococcal infections: options in 1989. Rev. Infect. Dis. **12**(Suppl. 6):S633–S644.
8. **National Committee for Clinical Laboratory Standards.** 1990. Development of in vitro susceptibility test criteria and quality control parameters. Tentative guideline M23-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **National Committee for Clinical Laboratory Standards.** 1990. Performance standards for antimicrobic disk susceptibility tests. Approved standard M2-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **National Committee for Clinical Laboratory Standards.** 1990. Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Roddy, R. E., H. H. Handsfield, and E. W. Hook III.** 1986. Comparative trial of single-dose ciprofloxacin and ampicillin plus probenecid for treatment of gonococcal urethritis in men. Antimicrob. Agents Chemother. **30**:267–269.
12. **Tegelberg-Stassen, M. J. A. M., J. C. S. van der Hoer, L. Mooi, J. H. T. Wagenvort, T. van Joost, M. F. Michel, and E. Stolz.** 1986. Treatment of uncomplicated gonococcal urethritis in men with two dosages of ciprofloxacin. Eur. J. Clin. Microbiol. **5**:244–246.