JOURNAL OF CLINICAL MICROBIOLOGY, May 1992, p. 1170–1173
0095-1137/92/051170-04$02.00/0

Vol. 30, No. 5

Interpretive Criteria for Susceptibility Testing of CI-960 (PD127391, AM-1091), Fleroxacin, Lomefloxacin, and Temafloxacin against *Neisseria gonorrhoeae*, Including Drug Stability in GC Agar Medium

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CI-960, fleroxacin, lomefloxacin, and temafloxacin were tested against over 100 strains of *Neisseria gonorrhoeae*. Each organism was tested in triplicate by using agar dilution and disk diffusion methods recommended by the National Committee for Clinical Laboratory Standards. CI-960 was the most potent compound, with a MIC against 90% of the strains tested of 0.008 μg/ml, and the least active was fleroxacin (MIC against 90% of strains, 0.12 μg/ml). Only the susceptible interpretive category was recommended for the CI-960 tests as follows: 5-μg disk, ≥39 mm (MIC correlate, ≤0.12 μg/ml). Three interpretive categories were proposed for the other fluoroquinolones as follows: fleroxacin, 5-μg disk susceptible at ≥33 mm (MIC correlate, ≤0.25 μg/ml), intermediate at 28 to 32 mm (MIC correlate, 0.5 μg/ml), and resistant at ≤27 mm (MIC correlate, >0.5 μg/ml); lomefloxacin, 10-μg disk susceptible at ≥35 mm (MIC correlate, ≤0.12 μg/ml), intermediate at 28 to 34 mm (MIC correlates, 0.25 to 0.5 μg/ml), and resistant at ≤27 mm (MIC correlate, >0.5 μg/ml); and temafloxacin, 5-μg disk susceptible at ≥36 mm (MIC correlate, ≤0.06 μg/ml), intermediate at 28 to 35 mm (MIC correlates 0.12 to 0.25 μg/ml), and resistant at ≤27 mm (>0.25 μg/ml). Interpretive agreement between disk diffusion results and the MICs was 100% for each agent, with the exception of lomefloxacin, which had a 0.9% minor error. All drugs were stable in GC agar medium for at least 21 days when stored at 2 to 5°C.

With the increasing prevalence of *Neisseria gonorrhoeae* strains resistant to standard therapies (3, 4), alternative regimens and drugs must be considered. This is especially true in certain endemic areas with a higher frequency of these resistant strains (4). The efficacy of using fluorinated quinolones, i.e., ciprofloxacin, norfloxacin, and rosoxacin, as alternatives in the treatment of uncomplicated gonococcal infections has been documented for more than 6 years (5, 20, 21). In fact, the Centers for Disease Control and The Medical Letter have recently listed norfloxacin and/or ciprofloxacin as an optional drug for the treatment of various *N. gonorrhoeae*-caused infections (3, 23). Four newer quinolones have also demonstrated remarkable in vitro potency against *N. gonorrhoeae*. These drugs include CI-960 (1), fleroxacin (8, 11), lomefloxacin (15), and temafloxacin (10). Two of the compounds, fleroxacin and temafloxacin, have been used successfully in the clinical treatment of uncomplicated gonorrhea (13, 19). Tentative interpretive criteria for these four antimicrobial agents have already been established for the nonfastidious, rapidly growing bacteria (2, 8, 15, 16). This report summarizes the study results used to develop interpretive criteria for the susceptibility testing of *N. gonorrhoeae* against these four cited fluoroquinolones. Stability of each compound in GC agar was also determined. The methods and study design conformed to those previously described by Jones et al. (17, 18) and the National Committee for Clinical Laboratory Standards (NCCLS) (25–27).

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MATERIALS AND METHODS

Antimicrobial agents. Reagent standard powders were supplied as follows: CI-960 was from Warner-Lambert/Parke-Davis (Ann Arbor, Mich.), fleroxacin was from Hoffmann-La Roche, (Nutley, N.J.), lomefloxacin was from G. D. Searle and Co. (Skokie, Ill.), and temafloxacin was from Abbott Laboratories (North Chicago, Ill.). Each antibiotic was also tested by 5-μg disks, except for lomefloxacin, which had a disk content of 10 μg. These disks were commercially prepared by Becton Dickinson Microbiology Systems (Cockeysville, Md.) or Difco Laboratories (Detroit, Mich.).

Organisms. The 103 or 104 (lomefloxacin only) strains of *N. gonorrhoeae* used for the determination of susceptibility breakpoints were selected to possess various drug resistances, as follows: penicillinase-producing *N. gonorrhoeae* (29 strains); penicillin-resistant, β-lactamase-negative isolates (38 or 39 strains); and penicillin-susceptible isolates (36 strains). Included in the penicillin-susceptible group were three strains with diminished susceptibility and in vivo response to fluoroquinolones (30). These were kindly provided by J. H. T. Wagenvoort of The Netherlands. All strains were confirmed to be gonococci by standard procedures.

Susceptibility testing methods. The susceptibility tests were performed by agar dilution and disk diffusion methods recommended by the NCCLS (26, 27). GC agar with 1% defined XV supplement devoid of cysteine (Prepared Media Laboratories, Tualatin, Oreg.) was used in all tests. Each of the isolates of *N. gonorrhoeae* was tested in triplicate by both the MIC and disk methods (25–27).

Statistical methods. The modal MIC and the mean inhibi-

TABLE 1. Regression statistics and proposed susceptibility criteria for CI-960, fleroxacin, lomefloxacin, and temafloxacin from studies of 103 to 104 *N. gonorrhoeae* strains

Antimicrobial agent (disk content [μg])	Regression formula (r)	Proposed zone diam (mm) (MIC correlate [μg/ml]) criteria:		
		Susceptible	Intermediate[a]	Resistant
CI-960 (5)	$y = 14.2 - 0.20x$ (0.66)	≥39 (≤0.12)	NA[b]	NA
Fleroxacin (5)	$y = 12.5 - 0.16x$ (0.73)	≥33 (≤0.25)	28–32 (0.5)	≤27 (>0.5)
Lomefloxacin (10)	$y = 13.5 - 0.18x$ (0.81)	≥35 (≤0.12)	28–34 (0.25–0.5)	≤27 (>0.5)
Temafloxacin (5)	$y = 11.2 - 0.17x$ (0.77)	≥36 (≤0.06)	28–35 (0.12–0.25)	≤27 (>0.25)
Enoxacin (10)[c]	NA	≥32 (≤0.5)	NA	NA
Ofloxacin (5)[c]	NA	≥31 (≤0.25)	NA	NA

[a] An intermediate or indeterminate result for an antimicrobial agent indicates a technical problem that should be resolved by repeated testing or by seeking more clinical experience in treating organisms with these zones or MICs (25–27).
[b] NA, not applicable (interpretive categories) or not available (regression formulae from NCCLS documents).
[c] Data from NCCLS M100-S3 (28).

tory zone diameter from the three datum points for each strain were used to calculate regression statistics. CI-960, fleroxacin, lomefloxacin, and temafloxacin MICs were compared with their zone diameters around the commercially prepared disks by regression analysis (adapted to computer). Suggested susceptible breakpoints conformed, where possible, to those of similar agents having NCCLS or published criteria (7, 28).

RESULTS AND DISCUSSION

Interpretive criteria for CI-960. Table 1 and Fig. 1 contain the regression line and interpretive criteria results for CI-960. This compound was the most potent of the four fluoroquinolones tested in this study against 103 strains of *N. gonorrhoeae* with a MIC for 90% of the strains tested (MIC_{90}) of 0.008 μg/ml and a range of ≤0.001 to 0.03 μg/ml. *N. gonorrhoeae* with MICs of 0.015 μg/ml or greater included the three strains from The Netherlands (30) with diminished susceptibility to fluoroquinolones and penicillin-resistant, β-lactamase-negative isolates. The 5-μg CI-960 disk zone diameters for all strains were ≥45 mm. The correlation coefficient for CI-960 zone diameters and MICs was poor (e.g., $r = 0.66$) because of a single population of very susceptible strains. Preliminary trials with healthy volunteers given 100- or 200-mg doses of CI-960 demonstrated the drug to be rapidly absorbed and well tolerated, and plasma concentrations remained above the MIC_{90}s required for most pathogens, including gonococci (6). With these favorable pharmacokinetics, we tentatively suggest a single susceptible interpretive category incorporating all CI-960 zone diameters and MICs. The criteria are ≥39 mm and ≤0.12 μg/ml to be classified as CI-960 susceptible. These recommendations may require revision as this compound is further developed.

Interpretive criteria for fleroxacin. Table 1 and Fig. 2 contain the fleroxacin interpretive criteria and MIC and zone diameter correlation results. Fleroxacin was active against the vast majority of gonococci tested, with a MIC_{90} of 0.12 μg/ml. However, the three strains from The Netherlands (30) had fleroxacin MICs of 1 μg/ml, which is threefold higher than the MIC_{90} for the remaining 100 strains tested. Because of this marked difference in organism population MICs, we believe that these strains should be considered resistant (ciprofloxacin therapy failures) until further experience with clinical and bacteriologic response rates for such strains are documented (30). With these data, and by also considering breakpoints for similar compounds recently listed in the NCCLS M100-S3 document (28), we suggest the following interpretive criteria for fleroxacin: susceptible at ≤0.25 μg/ml (≥33 mm), and resistant at >0.5 μg/ml (≤27 mm). Until the clinical responses are better characterized among

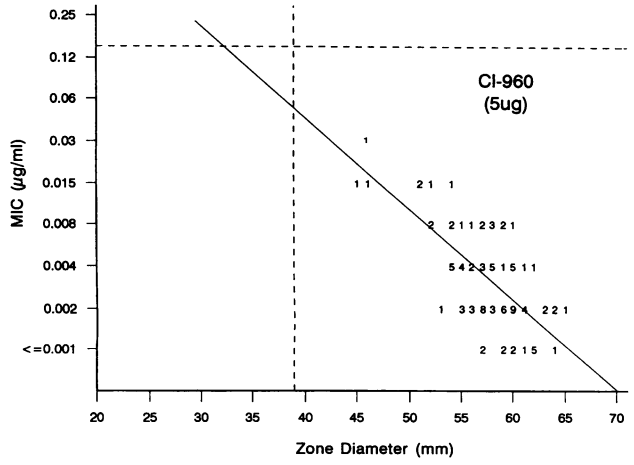

FIG. 1. CI-960 scattergram comparing 5-μg disk zone diameters with MICs. Vertical and horizontal broken lines are the proposed zone and MIC interpretive criteria, respectively, for this fluoroquinolone on the basis of limited clinical experience.

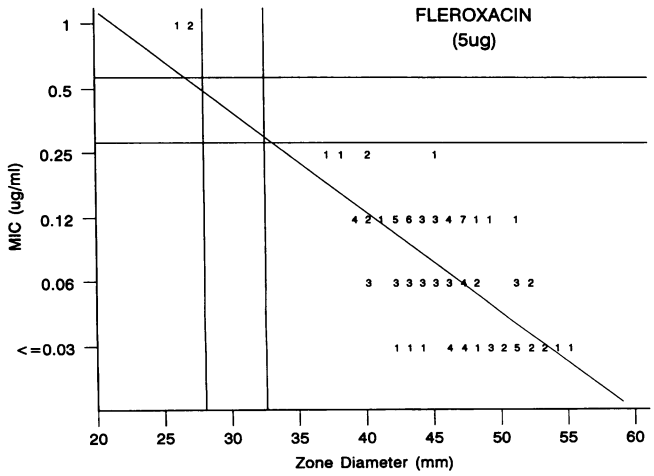

FIG. 2. Fleroxacin scattergram comparing 5-μg disk zone diameters with MICs. Vertical and horizontal lines are the proposed zone and MIC interpretive criteria, respectively.

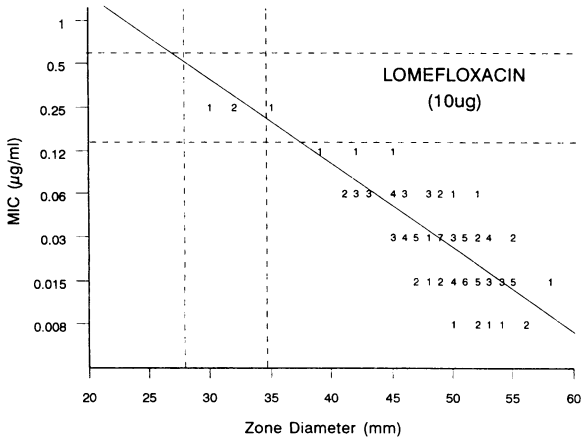

FIG. 3. Lomefloxacin scattergram comparing 10-µg disk zone diameters with MICs. Vertical and horizontal lines are the proposed zone and MIC interpretive criteria, respectively. The broken lines again indicate a drug for which there is limited clinical experience for this indication.

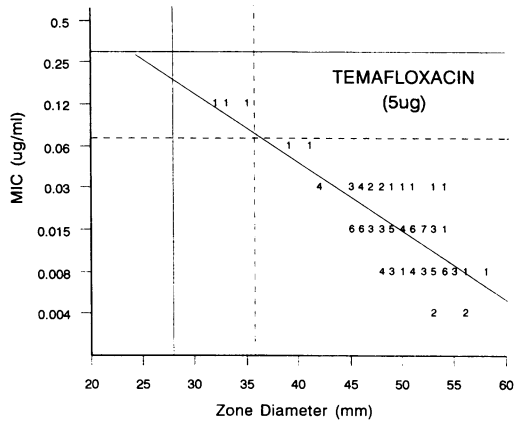

FIG. 4. Temafloxacin scattergram comparing 5-µg disk zone diameter with MICs. Vertical and horizontal broken lines are proposed modifications of zone and MIC interpretive criteria, respectively (28).

the strains with 0.5 µg of fleroxacin per ml MICs (none identified in this study), this MIC and zone diameter range (28 to 32 mm) will be designated as intermediate.

The acceptable clinical efficacy of fleroxacin for treating gonococcal urethritis was reported as early as 1988 by Lassus et al. in a study that compared a single 400-mg oral dose of fleroxacin to standard penicillin G therapy (19). More recently McCormack et al. (22) demonstrated that a single 400-mg fleroxacin dose was clinically comparable to a 200-mg ceftriaxone regimen, e.g., 319 of 322 patients were cured. However, the minor side effects (16%) in the fleroxacin treatment group were significantly ($P < 0.001$) greater than those encountered following cephalosporin therapy.

Interpretive criteria for lomefloxacin. The lomefloxacin MIC zone diameter scattergram and proposed interpretive criteria are found in Table 1 and Fig. 3. This fluoroquinolone was generally more active than fleroxacin, with a MIC_{90} of 0.06 µg/ml (MIC range, 0.008 to 0.25 µg/ml). All 104 strains of *N. gonorrhoeae* tested against the 10-µg lomefloxacin disks had zone diameters of ≥30 mm. At this time, and with no published clinical information for *N. gonorrhoeae* infection therapy, our proposed lomefloxacin breakpoints must be based on the criteria of the similar fluoroquinolones enoxacin, ofloxacin, and temafloxacin (28) and the available pharmacokinetics (24). However, because the lomefloxacin MICs for the ciprofloxacin refractory strains (MIC, 0.12 µg/ml) from the Netherlands (30) were 0.25 µg/ml, twofold higher than the MIC_{90} for the total collection of *N. gonorrhoeae* and considering the additional report of Putnam et al. (29) describing elevated lomefloxacin gonococcal MICs (1 µg/ml) from Southeast Asia strains, we tentatively propose three interpretive categories for lomefloxacin: susceptible at ≤0.12 µg/ml (disk zone diameter correlate, ≥35 mm), intermediate at 0.25 to 0.5 µg/ml (28 to 34 mm), and resistant at >0.5 µg/ml (≤27 mm). The intermediate category seems appropriate for these strains with lomefloxacin MICs of 0.25 µg/ml until the clinical trial results become known.

Interpretive criteria for temafloxacin. Table 1 and Fig. 4 present the results of interpretive criteria studies for temafloxacin. Temafloxacin was slightly more active than lomefloxacin against all 103 *N. gonorrhoeae* isolates (MIC_{90}, 0.03 µg/ml). The strains from the Netherlands (30), as with lomefloxacin, had twofold-higher temafloxacin MICs (0.12 µg/ml) compared with the MIC_{90}. Again, with these data and until more experience is forthcoming from clinical trials when treating these organisms with elevated temafloxacin MICs, we propose an intermediate category of 0.12 to 0.25 µg/ml (zone diameter correlate, 28 to 35 mm). This represents a proposed adjustment to the existing temafloxacin interpretive criteria published in the NCCLS M100-S3 document (28). Therefore, the suggested susceptible breakpoint for temafloxacin is ≤0.06 µg/ml (≥36 mm), the same as proposed for ciprofloxacin (7). No resistant strains (MICs, >0.25 µg/ml and ≤27 mm) were identified in this study. Quality control guidelines for testing *N. gonorrhoeae* were recently published in the NCCLS M100-S3 document (28).

Clinical experience with temafloxacin used for gonorrhea therapy was reported by Hook et al. (13). A single 200- or 400-mg oral dose of temafloxacin was found to be as effective as a 250-mg intramuscular dose of ceftriaxone, with bacteriologic cure rates of ≥98% (13). Our modified recommendations may require revision as more clinically resistant strains are identified.

Stability of the quinolones in GC agar. The four quinolones were tested in GC agar dilution plates stored at 5°C over a period of 21 days. Five replicate MIC tests of *N. gonorrhoeae* ATCC 49226 were made on days 0, 7, 14, and 21 of storage. All drug MICs remained constant as follows: CI-960, 0.002 µg/ml; fleroxacin, 0.03 µg/ml, lomefloxacin, 0.015 µg/ml; and temafloxacin, 0.015 µg/ml.

In summary, interpretive criteria were proposed for the four evaluated quinolones on the basis of in vitro data and, where available, on in vivo response considerations. Only a susceptible category was defined for CI-960 because of the current paucity of evaluable resistant strains (18). Fleroxacin activity against three strains of *N. gonorrhoeae* (30) tested in this study was diminished to an extent (MIC, 1 µg/ml) that a resistant interpretive category was also recommended (>0.5 µg/ml and ≤27 mm). Similarly, these Netherlands strains (30) had elevated lomefloxacin and temafloxacin MICs in contrast to the other recent *N. gonorrhoeae* isolates tested. Also, Southeast Asian isolates had lomefloxacin MICs significantly higher than those of the U.S. domestic strains (29), requiring consideration of three interpretive categories until more clinical information can be obtained. Interpretive agreement between disk diffusion results and the MICs for

each agent was 100%, except for lomefloxacin (99.1%). Lastly, we concur with the Centers for Disease Control (3) that newer fluoroquinolones should be considered as alternative antigonococcal therapy. However, recent reports of decreased susceptibility to ciprofloxacin (9, 14) and the existence of cross-resistance to quinolones among *N. gonorrhoeae* isolates (30) must be considered when choosing a broadly applicable therapeutic agent.

ACKNOWLEDGMENTS

We thank M. Barrett, B. Briggs-Gooding, and D. Johnson for their excellent technical support, S. Anderson for the statistical analyses, L. Miller for the word processing, and J. Wagenvoort for supplying some *N. gonorrhoeae* strains with diminished susceptibilities to fluoroquinolones.

This study was funded in part by technical grants from Warner-Lambert/Parke-Davis, Hoffmann-La Roche, G. D. Searle and Co., Lederle Laboratories, and Abbott Laboratories.

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