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Tentative Disk Diffusion Susceptibility Interpretive Criteria for Pefloxacin

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Standardized broth microdilution and disk diffusion susceptibility tests for pefloxacin were performed on 585 clinical isolates. The 5-μg pefloxacin disk is recommended, and the following breakpoints are proposed: susceptible, ≥19 mm (MIC, ≤2.0 μg/ml); resistant, ≤15 mm (MIC, >4.0 μg/ml); and intermediate, 16 to 18 mm.

Pefloxacin (1589 RB) is a fluorinated piperazinyl-substituted quinolone antimicrobial agent with the formula 1-ethyl-6-fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid. Pefloxacin has been shown to have good in vitro activity against most gram-negative species tested (including the *Enterobacteriaceae*, pseudomonads, and *Haemophilus* spp.) and against staphylococci (1, 3). Its in vitro activity is generally comparable to or slightly greater than that of enoxacin and norfloxacin. The present study evaluates 5-μg pefloxacin disks for disk diffusion susceptibility testing and makes tentative proposals for zone diameter interpretive criteria.

Pefloxacin was supplied as standardized powder by Rhone-Poulenc, Inc., Monmouth Junction, N.J. Comparative drugs were supplied by their respective manufacturers: norfloxacin, The Merck Institute, Rahway, N.J.; enoxacin, Parke-Davis, Div. Warner-Lambert Co., Ann Arbor, Mich.; ofloxacin, Ortho Pharmaceutical Corp., Raritan, N.J.; and ciprofloxacin, Miles Pharmaceuticals, Div. Miles Laboratories, Inc., West Haven, Conn. Disks containing 5 μg of pefloxacin and disks containing 10 μg of norfloxacin were prepared by BBL Microbiology Systems, Cockeysville, Md.

Dilution susceptibility tests were performed by the standardized microdilution method outlined by the National Committee for Clinical Laboratory Standards (6). Drugs were tested in serial twofold dilutions of cation-supplemented Mueller-Hinton broth at concentrations ranging from 0.03 to 16 μg/ml. When nonenterococcal streptococci and *Haemophilus* spp. were tested, the broth was supplemented with 3% lysed horse blood. For testing *Haemophilus* spp., 25 μg of NAD per ml was also added. Disk diffusion susceptibility tests were performed by the standardized method described by the National Committee for Clinical Laboratory Standards (5). Regression analyses correlating MICs with disk diffusion zone diameters were performed by the method of least squares. These data were plotted as scattergrams and evaluated for interpretive errors.

A total of 585 bacterial isolates (Table 1) were tested. Efforts were made to include quinolone-resistant isolates in proportions greater than those that occur in clinical experience for purposes of evaluating the disk test. Most of these were found among the pseudomonads, streptococci, and *Listeria monocytogenes*. The pefloxacin MICs inhibiting 90% (MIC_{90}s) of the isolates within each species are shown in Table 1, together with the MIC_{90}s of four comparative quinolones. Despite attempts to increase the proportion of resistant isolates, the MIC_{90}s rarely differed by more than one twofold concentration from those in previously published studies (1, 3).

Scattergrams of pefloxacin and norfloxacin MICs plotted against pefloxacin 5-μg disk diffusion zone diameters (Fig. 1) and norfloxacin 10-μg disk diffusion zone diameters (data not shown) were made. Norfloxacin was used as a control for the pefloxacin procedures, since its performance characteristics are well described (2). The regression formula for the norfloxacin data was essentially identical to that previously published (2). The application of National Committee for Clinical Laboratory Standards breakpoints (5) to the norfloxacin data yielded a minimal number of interpretive errors (7.3% minor errors, 0.2% very major errors, and no major errors).

For pefloxacin, ≤2.0 μg/ml was chosen as the susceptible MIC breakpoint, and >4.0 μg/ml was chosen as the resistant MIC breakpoint. These choices were based on somewhat scanty published pharmacokinetic data in humans indicating that a single oral dose of 400 mg of pefloxacin will achieve mean peak levels in plasma of 3.8 μg/ml (4). With comparable oral doses given every 12 h to two patients with meningitis, Wolff et al. found peak levels in plasma of 8.2 and 10.0 μg/ml after the third dose, with corresponding peak levels in cerebrospinal fluid of 3.8 and 3.0 μg/ml (7). The anticipated clinical oral doses of 600 and 800 mg, when given orally to male volunteers, resulted in maximal concentrations in plasma of 5.4 ± 1.0 and 6.06 ± 0.75 μg/ml, respectively (Bruce L. Moskovitz, personal communication). It appears, therefore, that until more pharmacokinetic data are available for the oral route, the MIC breakpoints selected above are reasonable.

The pefloxacin regression formula based on the 540 on-scale datum points in Fig. 1 and on a $\log_2 +9$ scale for the MIC (y axis) was $y = 16.39 - 0.34x$. The correlation coefficient was 0.90. Based on this regression formula, the zone diameter correlates for the aforementioned MIC breakpoints are as follows: susceptible, ≥18.7 mm; resistant, ≤15.8 mm. When rounded, the zone diameter breakpoints become as follows: susceptible, ≥19 mm; resistant, ≤15 mm; intermediate, 16 to 18 mm. With these breakpoints there were, among the 585 isolates shown in Fig. 1, 1 (0.2%) very major error, 1 (0.2%) major error, and 38 (6.5%) minor errors in interpretation. We therefore conclude

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TABLE 1. comparison of pefloxacin MIC_{90}s with those of four other quinolones against 585 clinical bacterial isolates

Organism (no. tested)	MIC_{90} (μg/ml) of:				
	Pefloxacin	Norfloxacin	Enoxacin	Ofloxacin	Ciprofloxacin
Haemophilus influenzae[a] (46)	0.06	0.06	0.125	0.06	≤0.03
Branhamella catarrhalis (18)	0.25	0.25	0.25	0.125	≤0.03
Citrobacter diversus (10)	0.125	0.06	0.25	0.06	≤0.03
Citrobacter freundii (10)	1.0	0.5	0.5	0.5	0.125
Enterobacter aerogenes (20)	0.25	0.25	0.25	0.25	≤0.03
Enterobacter agglomerans (10)	4.0	4.0	8.0	2.0	0.125
Enterobacter cloacae (21)	0.5	0.5	1.0	0.5	0.125
Escherichia coli (34)	0.5	0.5	1.0	0.25	0.06
Klebsiella spp.[b] (32)	1.0	1.0	1.0	1.0	0.125
Morganella morganii (10)	0.25	0.125	0.25	0.125	≤0.03
Proteus mirabilis (25)	0.25	0.125	0.5	0.125	≤0.03
Proteus vulgaris (10)	0.25	0.125	0.5	0.25	0.06
Providencia rettgeri (10)	0.25	0.25	0.5	0.5	0.06
Providencia stuartii (24)	2.0	4.0	4.0	4.0	1.0
Serratia marcescens (24)	1.0	1.0	0.5	0.5	0.25
"*Acinetobacter anitratus*" (15)	2.0	>16	16	2.0	2.0
Pseudomonas aeruginosa (55)	16	4.0	8.0	8.0	2.0
Pseudomonas spp.[c] (32)	8.0	>16	16	8.0	8.0
Staphylococcus aureus[d] (59)	0.5	2.0	2.0	0.5	0.5
Coagulase-negative *Staphylococcus* spp.[e] (25)	2.0	2.0	2.0	1.0	0.5
Listeria monocytogenes (10)	8.0	8.0	8.0	4.0	2.0
Enterococcus spp.[f] (25)	8.0	8.0	8.0	4.0	2.0
Streptococcus agalactiae (20)	16	8.0	>16	2.0	1.0
Streptococcus pneumoniae (20)	8.0	16	16	2.0	2.0
Streptococcus pyogenes (20)	>16	>16	>16	4.0	2.0

[a] Includes 22 β-lactamase-producing strains.
[b] Includes 7 *K. oxytoca* and 25 *K. pneumoniae* strains.
[c] Includes 3 *P. acidovorans*, 4 *P. cepacia*, 5 *P. fluorescens*, 6 *P. maltophilia*, 5 *P. putida*, and 9 *P. stutzeri* strains.
[d] Includes 36 β-lactamase-producing strains, of which 10 were methicillin resistant.
[e] Includes 17 β-lactamase-producing strains, of which 8 were methicillin resistant.
[f] Includes 1 *E. faecium* and 24 *E. faecalis* strains.

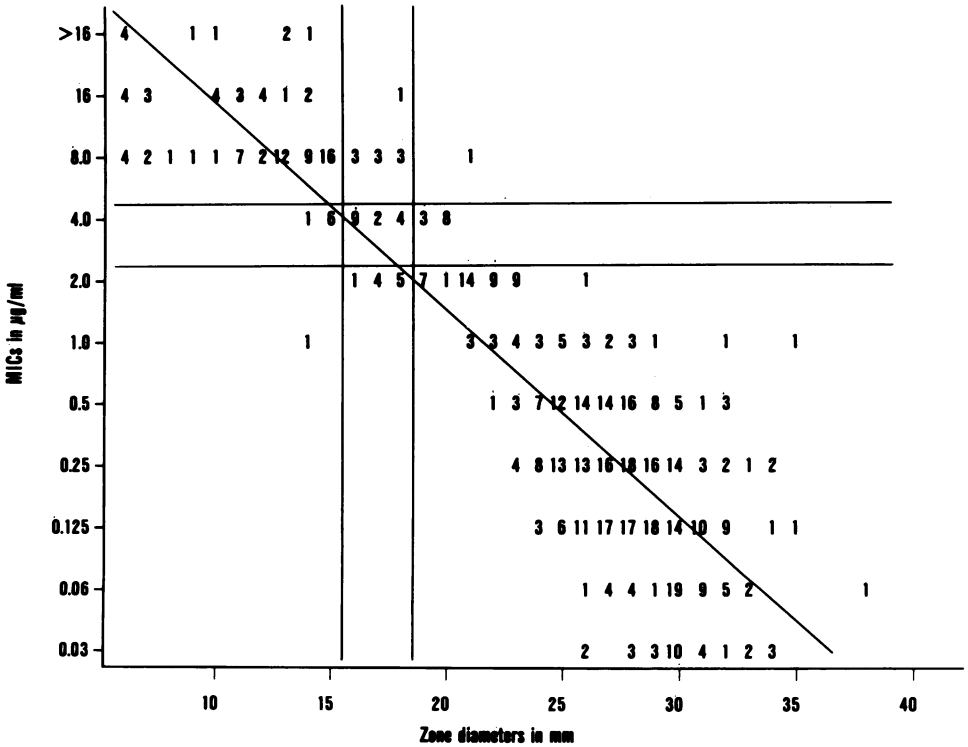

FIG. 1. Scattergram of pefloxacin MICs and 5-μg disk zone diameters for 585 bacterial isolates. Horizontal and vertical lines indicate proposed MIC and zone diameter breakpoints, respectively. The diagonal line is the regression line (slope, −0.34).

that the above zone diameter criteria may be applied tentatively to tests with the 5-μg pefloxacin disk, pending confirmation of the applicability of the MIC breakpoints.

LITERATURE CITED

1. **Clarke, A. M., S. J. V. Zemcov, and M. E. Campbell.** 1985. In vitro activity of pefloxacin compared to enoxacin, norfloxacin, gentamicin and new β-lactams. J. Antimicrob. Chemother. **15**:39–44.
2. **Jones, R. N., and A. L. Barry.** 1983. Norfloxacin (MK-0366, AM-715): in vitro activity and cross-resistance with other organic acids including quality control limits for disk diffusion testing. Diagn. Microbiol. Infect. Dis. **1**:165–172.
3. **Ligtvoet, E. E. J., and T. Wickerhoff-Minoggio.** 1985. In vitro activity of pefloxacin compared with six other quinolones. J. Antimicrob. Chemother. **16**:485–490.
4. **Montay, G., Y. Goueffon, and F. Roquet.** 1984. Absorption, distribution, metabolic fate, and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys, and humans. Antimicrob. Agents Chemother. **25**:463–472.
5. **National Committee for Clinical Laboratory Standards.** 1984. Performance standards for antimicrobic disk susceptibility disk. Approved standard, M2-A3, 3rd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
6. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Wolff, M., B. Regnier, C. Daldoss, M. Nkam, and F. Vachon.** 1984. Penetration of pefloxacin into cerebrospinal fluid of patients with meningitis. Antimicrob. Agents Chemother. **26**:289–291.