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Cefoperazone Disk Diffusion Susceptibility Test: Confirmation of the Tentative Interpretive Criteria, *Pseudomonas aeruginosa* Cross-Resistance, and Determination of Quality Control Performance Limits

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Cefoperazone disk diffusion test and minimum inhibitory concentration comparison studies were performed on 421 recent bacterial isolates, using 30- and 75-μg commercially prepared disks. Acceptable correlation coefficients (−0.82 to −0.86) and very major (false-susceptible) interpretive error rates (<1%) were obtained with both disk concentrations. The interpretive criteria for both disks were identical. Using the preferred 75-μg disk, the Thornsberry et al. criteria (J. Clin. Microbiol. **15**:769–776, 1982) of ≥18 mm = susceptible (≤32 μg/ml) and ≤14 mm = resistant (>64 μg/ml) resulted in only 5.5% of strains having indeterminate-range zone diameters; the 30-μg disk had 6.9% of strains with indeterminate zone diameters. The 75-μg disk, excluding the testing of enterococci, minimized the very major and other interpretive errors to <5%. Larger zone diameters will contribute few technical problems with either disk concentration. Data from 1,320 zone diameters submitted for each quality control strain indicated no significant ($P > 0.05$) difference between disks made by the three major manufacturers, and consistent results were obtained within each laboratory with numerous lots of Mueller-Hinton agar (except for one manufacturer). Individual daily test and accuracy quality control ranges were calculated from clinical investigator laboratory data at 16 hospitals based on mean zone sizes and from an additional 8 laboratories with both mean and median calculations. The quality control data were nearly identical, and ranges calculated by the two methods were very similar. Susceptibility tests of *Pseudomonas aeruginosa* indicate that the cefoperazone disk or minimum inhibitory concentration test would accurately predict *P. aeruginosa* susceptibility test results for other pseudomonas-active cephalosporins (cefsulodin and ceftazidime), thus producing no very major interpretive errors.

Cefoperazone sodium (formerly T-1551) is a very broad-spectrum semisynthetic cephalosporin reported to have inhibitory effects against the *Enterobacteriaceae*, staphylococci, non-enterococcal *Streptococcus* spp., *Pseudomonas aeruginosa*, *Haemophilus* spp., *Neisseria* spp., and some anaerobes (1, 6, 12, 15, 17, 23, 29, 33). Its in vitro activity has been judged to be significantly superior to commonly used parenteral cephalosporins such as cephalothin, cephapirin, cefamandole, cefazolin, and cefoxitin. When cefoperazone was compared with other new investigational (third-generation) cephalosporins, its activity was found to be comparable, particularly against the more commonly isolated bacterial pathogens, including *P. aeruginosa* (1, 6, 10–17, 22–24, 30–33). A few beta-lactamases of gram-negative bacterial origin can hydrolyze cefoperazone at rates faster than those reported for cefotaxime (HR756), moxalactam (LY127935, 6059-S), ceftazidime (GR20263), ceftizoxime (FK749), cefmenoxime (SCE 1365), or HR221 (11, 16, 17, 22–24, 30). Cefoperazone hydrolysis rates are most comparable to cefamandole (enzyme type variable), yet cefoperazone appears to possess a greater potential clinical spectrum of activity than does cefamandole or other currently used cephalosporins.

In this report we present data from several recent studies that compare commercially pre-

pared cefoperazone 30- and 75-μg disk zone diameters with the reference cefoperazone minimum inhibitory concentrations (MICs) as determined in divalent cation-supplemented Mueller-Hinton broth (21). These results were compared with those published by Thornsberry and colleagues and by Welch et al. (29, 33). Additional quality control parameters for both cefoperazone disk concentrations were established by multilaboratory clinical trials, using numerous disk and agar media preparations. Cross-resistance studies compared cefoperazone with the other cephalosporins (cefsulodin and ceftazidime) active against *P. aeruginosa* and correlated discrepant results with beta-lactamase hydrolysis of the substrates.

MATERIALS AND METHODS

Antimicrobial agents and media. Cefoperazone and carbenicillin were obtained from Pfizer Inc., New York, N.Y., and the cefoperazone 30- and 75-μg disks were from BBL Microbiology Systems, Cockeysville, Md. The cefsulodin powder was provided by Abbott Laboratories, North Chicago, Ill.; the ceftazidime and nitrocefin were provided by Glaxo Research Group Limited, Greenford, U.K.; and PADAC (pyridine-2-azo-*p*-dimethylaniline cephalosporin) was from Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J. All compounds were diluted in divalent cation (25 mg of magnesium and 50 mg of calcium per liter)-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (21). A 16-dilution schedule was utilized for each drug, ranging from 0.008 and 256 μg/ml.

For the disk zone quality control study, three lots of 30- and 75-μg cefoperazone disks were used, one each from BBL, Difco, and Pfizer (Groton, Conn.). All lots had assayed potencies ranging from 97 to 122% of target value by microbiological assays. The potency range for UV methods was 90 to 120%.

Bacterial strains. The organisms used for the regression line phase of the studies were 421 isolates collected from the clinical microbiology laboratories of the collaborating investigators and others contributed by P. C. Fuchs (St. Vincent Hospital and Medical Center, Portland, Ore.), E. H. Gerlach (St. Francis Hospital, Wichita, Kans.), and H. M. Sommers (Northwestern Memorial Hospital, Chicago, Ill.). The strains were representative of typical clinical isolates collected in 1979 to 1980 except for some *Pseudomonas* spp. (not *P. aeruginosa*) and some fastidious drug-resistant *Streptococcus* spp. strains that come from the Centers for Disease Control collection (C. Thornsberry). These strains were further subcategorized into the following species for genus groupings: 15, *Acinetobacter calcoaceticus* subsp. *anitratus*; 10, *Citrobacter diversus*; 11, *C. freundii*; 19, *Enterobacter aerogenes*; 9, *E. agglomerans*; 16, *E. cloacae*; 2, *E. gergoviae*; 25, *Escherichia coli*; 26, *Klebsiella pneumoniae*; 10, *Morganella morganii*; 25, *Proteus mirabilis*; 8, *P. vulgaris*; 10, *Providencia rettgeri*; 19, *P. stuartii*; 3, *Pseudomonas acidovorans*; 50, *P. aeruginosa*; 3, *P. cepacia*; 6, *P. fluorescens*; 3, *P. maltophilia*; 5, *P. putida*; 9, *P. stutzeri*; 25, *Serratia marcescens*; 48, *Staphylococcus aureus*; 25, *Streptococcus faecalis*; 19, *S. pneumoniae*; and 20, *S. pyogenes*.

Quality control investigations. Those strains recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for quality control of the disk diffusion test (20) were sent as lyophilized disks to each of eight participating institutions. These strains were *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853. The participating investigators and medical centers in the quality control protocol were the four authors and P. C. Fuchs, E. H. Gerlach, J. M. Matsen (University of Utah Medical Center, Salt Lake City, Utah), and L. B. Reller (University of Colorado Medical Center, Denver, Colo.). Each investigator performed 50 tests for each quality control organism on a lot of agar unique to that facility (nine total agar lots from four manufacturers) and 5 tests on a Mueller-Hinton agar lot common to all investigators. The total number of zones reported for each disk and control organism was 1,320. These data were statistically analyzed to detecting variation in the disk lots (six), agar lots (nine from four sources), or investigator by methods previously described (9, 20).

Clinical investigators contributing in vitro studies to Pfizer Inc. used the NCCLS disk method, 75-μg cefoperazone disks, and the three recommended quality control organisms (20). These data (16 hospitals) were pooled from the quality control strains and statistically analyzed by commonly used computer programs (9). No common lot of agar was used in this phase to monitor interlaboratory variations.

Antibiotic susceptibility tests. MICs were determined by the broth microdilution method. The test trays were prepared commercially (Prepared Media Laboratory, Portland, Ore.) with a single lot of Mueller-Hinton broth and were distributed to the testing laboratories. These trays were stored at −20°C or below until inoculated. Before use, the trays were thawed to room temperature (ca. 20 to 30 min) and inoculated with disposable inoculators delivering ca. 5 μl of inoculum to each well. The final inoculum achieved was 1×10^5 to 5×10^5 colony-forming units per ml. For the testing of fastidious streptococci including *S. pyogenes* and *S. pneumoniae*, the inoculum was standardized in Mueller-Hinton broth containing 5% lysed rabbit erythrocytes, and 0.1 ml of this adjusted cell suspension was added to each microdilution well, giving a final concentration of ca. 10^5 colony-forming units per ml. The MIC was recorded as the lowest concentration totally inhibiting visible bacterial growth (clear well) after approximately 15 to 18 h of incubation at 35°C.

The correlative disk diffusion susceptibility results in millimeters were determined by the method of Bauer and colleagues as modified by the NCCLS (4, 20). These data were compared with the MICs by the method of least squares as adapted to computers and the techniques described by Metzler and DeHaan (19).

Beta-lactamase hydrolysis test were performed with chromogenic cephalosporin substrates (nitrocefin and PADAC), and resultant data were correlated to MICs for cefoperazone, cefsulodin, and ceftazidime obtained by standardized methods (25, 27). UV spectrophotometric procedures have been previously described (11).

RESULTS

The cefoperazone disk diffusion test regression line studies were performed on 421 recent

TABLE 1. Regression line statistics for the cefoperazone 30- and 75-μg disks and various regression intervals, eliminating the parabolic segment of the curve below 1- to 2-μg/ml cefoperazone MICs

Cefoperazone disk content (μg)	Regression interval (μg/ml)	Correlation coefficient	Slope	y intercept (μg/ml)	Total no. of observations
30	0.06–256	−0.86	−0.40	19.4 (1,442)	408
	2.0–256	−0.80	−0.30	18.2 (594)	204
	4.0–64	−0.77	−0.25	17.2 (305)	54
75	0.06–256	−0.82	−0.44	21.2 (4,989)	402
	2.0–256	−0.75	−0.31	19.1 (1,135)	208
	8.0–128	−0.71	−0.21	17.9 (474)	102

bacterial isolates, using 30- and 75-μg commercial disks (Table 1; Fig. 1 and 2). These strains were different from those used in an earlier report on the cefoperazone disk test (29). The regression line statistics were calculated over three intervals to show correlations for the entire range and those excluding the parabolic segment with cefoperazone MICs below 2.0 μg/ml. Correlation coefficients varied from −0.71 to −0.86, with 402 and 408 strains of the 421 strains contributing to the full-scale regression analysis. These coefficients may be considered low but represent combined, yet controlled, data from three laboratories, using different lots of Mueller-Hinton agar and different technical staffs. The regression lines and scattergram plots

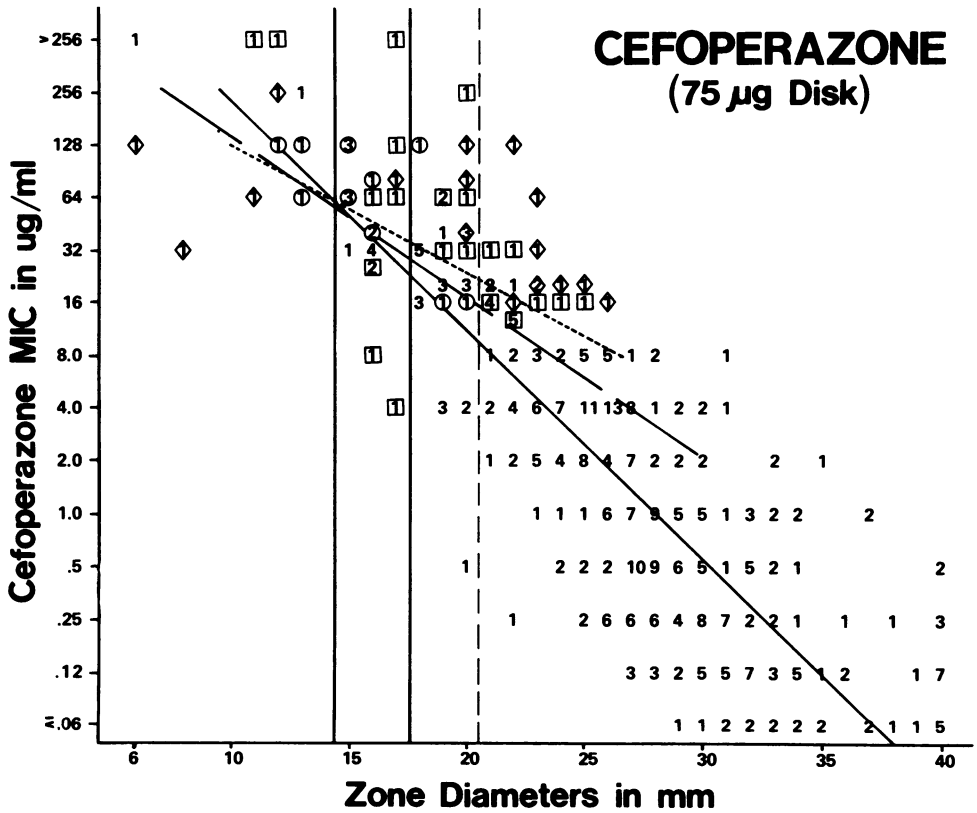

FIG. 1. Scattergram showing the correlation of 75-μg cefoperazone disk zones with MICs determined by reference broth microdilution methods. Three regression lines are plotted ranging from 0.06 to 256 (——)-, 2.0 to 256 (——)-, and 8.0 to 128 (-----)-μg/ml MICs. The identity of the organisms having MICs of ≥16 μg/ml and with zones in the indeterminate or resistant categories are shown as follows: □, *Enterobacteriaceae*; ◇, *Pseudomonas* spp.; ○, *Acinetobacter* spp.; no surrounding symbol, *Enterococcus*. All other unidentified data points are *S. aureus*, *Enterobacteriaceae*, and non-enterococcal *Streptococcus* spp. Vertical lines are the interpretive criteria of Thornsberry et al. (29), with the broken line representing possible application of the 75-μg disk to a ≤16-μg/ml susceptible breakpoint.

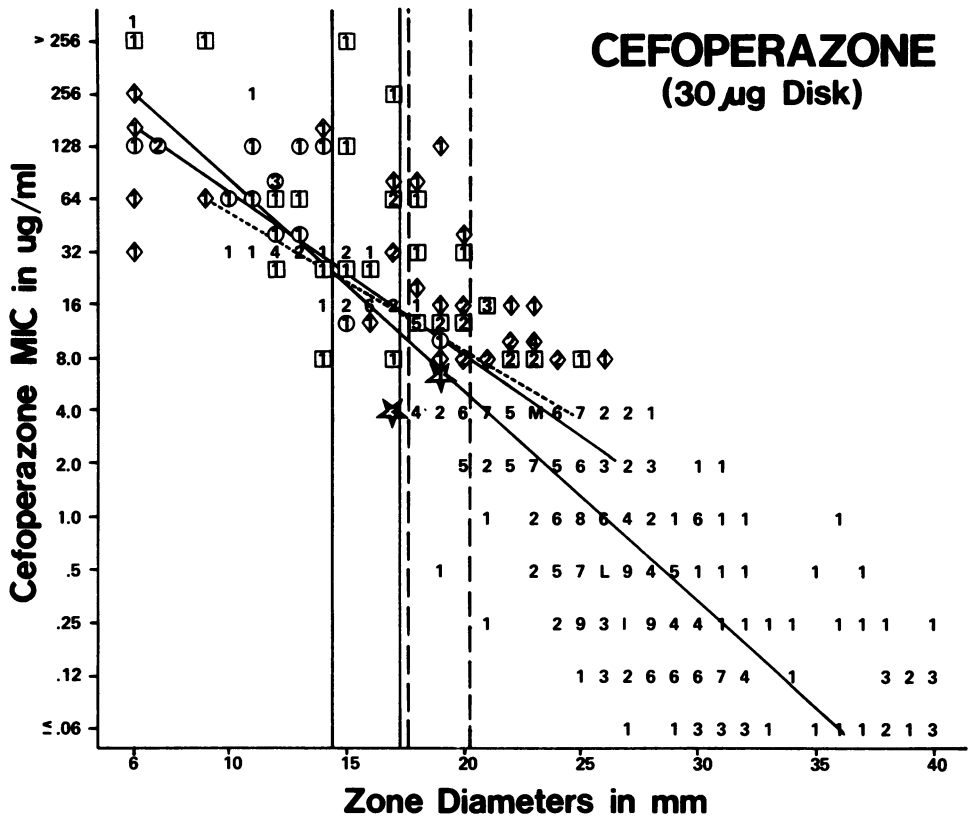

FIG. 2. Scattergram showing the correlation of 30-µg cefoperazone disk zone diameters to MICs. Three regression lines are plotted ranging from 0.06 to 256 (——)-, 2.0 to 256 (——)-, and 4.0 to 6.0 (-----)-µg/ml MICs. The identity of all strains with MICs of ≥8 µg/ml and having zones within the indeterminate or resistant categories are shown with the same symbols as Fig. 1. A star (☆) is the symbol used for *S. aureus* isolates. The solid vertical lines are the suggested interpretive criteria of Thornsberry et al., with the broken lines representing possible interpretive breakpoints with ≤8 µg/ml as a susceptible correlate cefoperazone MIC (29).

are found in Fig. 1 and 2. The solid vertical lines are those interpretive criteria suggested by Thornsberry et al. for each disk potency (29). In Fig. 1 (75-µg disk) the short-interval regression line of 2.0 to 256 µg/ml correlates well with the previously established criteria, using a correlative susceptible cefoperazone MIC of ≤32 µg/ml and a resistant concentration of >64 µg/ml. The interpretive error rates would be: very major (false-susceptible) = 0.95%; major (false-resistant) = 0.23%; and minor error = 5.46%. Figure 2 presents the 30-µg cefoperazone disk statistics, including three regression lines and two interpretive criteria sets. Using ≤16 µg/ml as the susceptible cefoperazone level, the cefoperazone 30-µg disk may be used with some confidence. The criteria of Thornsberry and colleagues (solid vertical lines) correlates well with these data, producing interpretive error rates of: very major = 0.71%, major = 0.48%, and minor = 9.26%. If the susceptible category was redefined as an MIC of ≤8 µg/ml, the interpretive zone diameters would be: susceptible, ≥21 mm; and resistant, ≤17 mm. These criteria were calculated by using the regression lines for MIC intervals of 2.0 to 256 and 4.0 to 64 µg/ml, plus the application of the error rate bounding method of Metzler and DeHaan (19). The later criteria produce a Zs of 21 mm and a Zr equaling Zs.

The use of the various susceptible cefoperazone MICs results in generally good disk test correlation statistics for both cefoperazone disk concentrations. Using the 75-µg disk criteria of ≥18 mm = susceptible (≤32 µg/ml) and ≤14 mm = resistant (>64 µg/ml), only 5.5% of the 421 strains had zone diameters in the indeterminate range (29). Among these 23 strains in the indeterminate zone range, 11 were *A. calcoaceticus* subsp. *anitratus*, 6 were *Enterobacteriaceae*, 5 were enterococci, and only 1 was *Pseudomonas* spp. Thus, it appears that only rarely encountered gram-negative bacilli and enterococci dominate this population of zone diameters. As the cefoperazone-susceptible MIC was reduced

to ≤16 or ≤8.0 μg/ml, the need for a 75-μg disk concentration and resulting larger zone diameters was also diminished. The 30-μg disk breakpoint criteria of ≥18 mm for susceptible (MIC, ≤16 μg/ml) and ≤14 mm for resistant (MIC, >32 μg/ml) resulted in only 6.9% of the zones in the indeterminate area (29). These strains included 13 enterococci, 8 *Enterobacteriaceae*, 5 nonenteric gram-negative bacilli, and 3 *S. aureus*. The acinetobacters that were indeterminate by the 75-μg disk were classified as resistant (≤14 mm) by the 30-μg disk. If the susceptible MIC breakpoint were further reduced to ≤8.0 μg/ml, >40% of the *S. aureus* isolates would be miscategorized as being resistant or indeterminate, although most had susceptible cefoperazone MICs of 2.0 and 4.0 μg/ml. The false-susceptible rate, however, would be nil, but the major and minor errors would be increased to >12%.

Another consideration about disk concentration is the incidence of large zone diameters, especially >35 mm. These diameters may interfere with the interpretation of adjacent disk zones. For the 30- and 75-μg cefoperazone disks, only 5.2 and 6.7% of zones were >35 mm, respectively. *Streptococcus pneumoniae* in this protocol accounted for 17 of 22 larger zones for the 30-μg disk and 19 (all pneumococcus strains tested) of 28 zones by the 75-μg disk. We believe large zones will present few problems for the user of either cefoperazone disk content.

Cephalosporins have not been widely used for the treatment of enterococcal or other serogroup D streptococcal infections. Therefore, an analysis of disk data excluding the enterococci as a potentially tested or treatable pathogen might be informative. Although cefoperazone is more active than most other cephalosporins against *Streptococcus faecalis*, these strains should not be considered currently as treatable pathogens. With the 30-μg disk, 96% (24 of 25) of the enterococcus strains were either resistant or indeterminate. Using the 75-μg disk, 72% of these strains would be classified as susceptible, clearly a false value in the absence of substituting clinical studies. If a 75-μg disk were used, serogroup D streptococci should not be tested. Exclusion of the enterococci from our data analysis would result in a further reduction of interpretive errors, with only 2.3 and 4.5% of zones in the resistant and indeterminate zones, respectively. A second option would be to test the enterococci, but use the 30-μg disk (29) that would more correctly categorize these organisms. In a large study of cefoperazone MICs, 6.8% of strains tested by the laboratories were *Streptococcus faecalis*, and nearly all were from the urinary tract (12). The final choice must await the treatment efficacy results of cefoperazone on well-documented *Streptococcus faecalis* and other bacterial infections with MICs of 16 or 32 μg/ml.

Cefoperazone is also well known to have susceptibility to some beta-lactamases (1, 23). A number of strains with known beta-lactamase production were tested for cefoperazone hydrolysis and then for susceptibility by MIC and disk methods. In every instance where a significant hydrolysis was found by standard methods (see Materials and Methods), a resistant or indeterminate zone or MIC was also noted. This was particularly true for the type III-TEM beta-lactamase found in numerous gram-negative bacilli, some of which readily destroy cefoperazone. An examination of the previously reported susceptibility data from clinical *Enterobacteriaceae* species (12) shows that at a susceptible MIC breakpoint of ≤16 μg/ml, 58% of all cefoperazone-resistant isolates were *E. coli*. Other prevalent resistant organisms were: *Enterobacter cloacae* and *Serratia marcescens*, 6.9% each; *C. freundii*, 5.8%; and *Klebsiella* spp., 5.8%. Those strains resistant to cefoperazone were only 3.4% of all the 5,503 *Enterobacteriaceae* previously tested (12).

Table 2 presents the results of two investigations of quality control for the standardized disk diffusion test (20). In an eight-laboratory controlled trial, methods were used as described by the NCCLS (20) and the study structure was as reported previously by Gavan et al. (9). The data from 1,320 zone diameters were submitted for each quality control strain and cefoperazone disk potency. No significant ($P > 0.05$) difference was encountered between disks manufactured by each of three companies. On a common lot of agar (Difco dry powder, lot 675407), participants had statistically similar results; thus, technical variation was considered minimal. Yet, two participants using agar from the same manufacturer (Oxoid Ltd., London, U.K.) reported zone diameters different from those submitted by other laboratories using Difco, BBL, and GIBCO Diagnostics (Madison, Wis.) Mueller-Hinton agars. The statistical analysis of all 1,320 zones for each disk concentration provided mean and median zones 1 mm different from those calculated after excluding data from participants using Oxoid agar. The individual daily test and accuracy control ranges calculated by the Gavan et al. method of medians and those computed with the more conventional mean ± 2 standard deviations method were nearly identical. These recommendations were further confirmed by the submitted quality control zones from 16 hospitals also contributing cefoperazone in vitro studies to Pfizer Inc. At the time of writing, at least 818 zones were reported for each NCCLS quality control strain. The mean zone ± standard deviation of each were as

TABLE 2. Recommended individual daily quality control limits and accuracy control parameters for the 30- and 75-μg cefoperazone disks and the three NCCLS quality control organisms (20)

Organism	Disk content (μg)	Mean	Individual daily test control zone diam (mm)	Accuracy control zone diam (mm)[a]	Precision control range[b] of 5 values	
					Maximum (mm)	Avg[c] (mm)
E. coli (ATCC 25922)	30	28.2	25–31 (24–32)[d]	26–30	7	3.5
		29.0	26–32 (26–32)[e]	27–31	7	3.5
	75	30.1	27–33 (26–34)[d]	28–32	7	3.5
		30.9	28–34 (28–34)[e]	29–33	7	3.5
		31.1	(27–35)[f]			
S. aureus (ATCC 25923)	30	26.7	24–30 (22–32)[d]	25–29	7	3.5
		26.1	23–29 (21–31)[e]	24–28	7	3.5
	75	29.1	26–32 (24–34)[d]	27–31	7	3.5
		28.3	25–31 (24–33)[e]	26–30	7	3.5
		29.8	(26–34)[f]			
P. aeruginosa (ATCC 27583)	30	22.8	20–26 (20–26)[d]	21–25	7	3.5
		23.2	20–26 (21–26)[e]	21–25	7	3.5
	75	25.4	22–28 (22–28)[d]	23–27	7	3.5
		25.8	23–29 (23–29)[e]	24–28	7	3.5
		26.1	(23–30)[f]			

[a] Mean of five values.

[b] Maximum value minus minimum value obtained in a series of five consecutive tests should not exceed the listed maximum limits; the mean should fall within the range under "accuracy control."

[c] In continuing series of ranges from consecutive groups of five tests each, the average range should approximate the listed value.

[d] Range as determined by the method described by Gavan et al. (9), with the second range calculated from the mean ± 2 standard deviations in parentheses.

[e] Range as determined by the method described by Gavan et al. (9), with the second range calculated from the mean ± 2 standard deviations in parentheses. Ranges representing those data excluding two media lots producing significantly different zones.

[f] Data from a 16-laboratory trial of cefoperazone clinical investigators.

follows: *S. aureus*, 29.86 ± 2.17 mm; *E. coli*, 31.06 ± 1.97 mm; and *P. aeruginosa*, 26.07 ± 1.74 mm. The means and medians were identical, and only the mean ± 2 standard deviation ranges were computed for comparison (see Table 2).

Previously published cross-resistance studies of cefoperazone and currently marketed cephalosporins such as cephalothin, cefamandole, and cefoxitin demonstrated the need for a separate disk or susceptibility test (29). The slight but significantly greater susceptibility of cefoperazone to *Enterobacteriaceae* beta-lactamase compared with cefotaxime-like methoximino cephalosporins or moxalactam also indicates a need for separate testing (C. Thornsberry, Drugs, in press). Here we present (Table 3) cross-resistance data for cefoperazone and the other two antipseudomonas cephalosporins, ceftazidime and cefsulodin. At least 93.7% total interpretive agreement was found among all three drugs. Only four strains were resistant to one or more of these cephalosporins. Of the six organisms that were resistant or indeterminate to cefoperazone, two were susceptible to cefsulodin inhibition. However, four isolates were susceptible (MICs, 2.0 to 8.0 μg/ml) to ceftazidime. The six strains were subjected to beta-lactamase hydrolysis studies. Results of these studies indicate that five of the six strains produced beta-lactamase as detected by chromogenic cephalosporin reagents (25, 27). The two strains resistant by MIC and disk tests to ceftazidime probably had permeability mutations since one produced no beta-lactamase and the other's beta-lactamase failed to hydrolyze ceftazidime over several hours. The remaining four strains all produced 4+ beta-lactamase (by chromogenic spot tests) that slowly hydrolyzed cefsulodin or cefoperazone or both at rates explaining their differential susceptibility (data not shown). For best predictive susceptibility test statistics, a cefoperazone disk or MIC test for cefsulodin and ceftazidime would produce no very major (false-susceptible) errors, 1.4 to 4.3% major errors (false-resistant), and only 2.8 to 5.8% minor errors. Using the cefsulodin or ceftazidime disks or MICs to predict the cefoperazone susceptibility could produce up to a 4.3% very major error in our series. The actual rate of *P. aeruginosa* resistance to cefoperazone (>16 μg/ml) in a series of 718 clinical isolates was only

TABLE 3. Cross-resistance analysis comparing the results of in vitro tests for the 50 *P. aeruginosa* strains used to determine the disk interpretive criteria and 19 additional drug-resistant isolates tested against the three cephalosporins having significant antipseudomonal activity

MIC (μg/ml)	% of isolates (no. of strains)					
	Ceftazidime MICs (μg/ml)			Cefsulodin MICs (μg/ml)		
	≤8	16	≥32	≤16	32	≥64
Cefoperazone						
≤16	91.3 (63)			91.3 (63)		
32	1.4 (1)[a]		1.4 (1)[a]	1.4 (1)		1.4 (1)
≥64	4.4 (3)[a]		1.4 (1)[a]	1.4 (1)	2.9 (2)	1.4 (1)
Cefsulodin						
≤16	94.2 (65)			X[b]	X	X
32	2.9 (2)			X	X	X
≥64			2.9 (2)	X	X	X

[a] Six strains were resistant or intermediate to one or more of the cephalosporins. A discussion of their beta-lactamase activity and enzyme hydrolysis rates is found in the text.

[b] X, Not applicable; direct comparison of same drug (cefsulodin).

7%, and for ceftazidime (>8 μg/ml) it was 4.8% among 1,153 strains (12; R. N. Jones et al., J. Antimicrob. Chemother., in press).

DISCUSSION

Cefoperazone possesses well-documented antimicrobial activity against the *Enterobacteriaceae*, staphylococci, non-enterococcal *Streptococcus* spp., *P. aeruginosa, Haemophilus* spp., *Neisseria* spp., and some anaerobes (1, 6, 12, 15, 17, 23, 29–33). This spectrum of inhibition is significantly superior to cephalothin, cefoxitin, and cefamandole to support the need for separate susceptibility testing (29). Clearly, the development of a reliable cefoperazone disk diffusion test is a prime consideration since the majority of clinical laboratories worldwide use that procedure. Data presented here and earlier by Thornsberry et al. and Welsh et al. show remarkable similarities in recommendations and interpretive criteria (29, 33). These studies have used several disk concentrations to formalize sets of tentative standards for the 30- and 75-μg disks. The human pharmacology and ultimate choice of the susceptible cefoperazone MIC correlate have a critical bearing on the ultimate decision.

The correlation of cefoperazone human pharmacokinetics, susceptible MIC criteria, proposed interpretive zone diameters (20, 29), and interpretive error rates is found in Table 4. Two proposed intravenous dose schedules produce high and prolonged cefoperazone serum concentrations that are in excess of several possible susceptible MICs for 25 to 75% of the dose interval (2, 5, 18, 28). Similarly, cefamandole and cefotaxime have levels above their susceptible MICs for 30 to 45% of the dose interval. However, the latter two drugs require more frequent infusions and generally larger daily doses to achieve statistics comparable to that of cefoperazone. The ≤16- and ≤32-μg/ml susceptible breakpoints are those previously cited by Thornsberry et al., and the lower ≤8.0-μg/ml level is a new data treatment for comparison with cefamandole and cefotaxime (8, 20, 29). The disk interpretive zones suggested here and by others result in very acceptable error rates, with only the cefamandole 30-μg disk having >1% false-susceptibles (8, 20, 29, 33). The cefoperazone blood levels with 2- and 1-g intravenous infusions appear to support MIC susceptible correlates for ≤32 and ≤16 μg/ml, respectively (2, 5, 18, 28). Disk development data also dictate that, if a ≤32-μg/ml susceptible MIC were chosen, then a 75-μg/ml disk must be utilized to produce acceptable predictive zones through the critical segment of the regression line. However, if the lower concentration (≤16 μg/ml) were to be applied, then a 30-μg cefoperazone disk would be desirable. In our opinion, the ≤8.0-μg/ml breakpoint MIC is ultraconservative and a poor choice. The final choice may well rest with the clinical response data of bacterial strains with cefoperazone MICs of 8, 16, and 32 μg/ml compared with the more common MICs of ≤2.0 μg/ml. We further recommend that enterococci not be tested for cefoperazone or any other cephalosporin susceptibility, particularly with the 75-μg cefoperazone disk. Eliminating this oganism from testing would result in a marked reduction of interpretive test errors and the incidence of clinically misleading data. The 30-μg cefoperazone disk more correctly categorized enterococcal susceptibility as being among the resistant and intermediate strains. Unlike cefotaxime and moxalactam, cefoperazone does not have a large population of MICs within an indeterminate (moderately suscepti-

TABLE 4. Comparison of in vivo pharmacology and in vitro testing[a]

In vivo pharmacology		In vitro testing statistics							
Cephalosporin/dosage	% Dosage interval above susceptible MIC	MIC criteria (μg/ml)		Cephalosporin disk content (μg)	Zone diam criteria (mm)		% Interpretive errors		
		Susceptible	Resistant		Susceptible	Resistant	Very major	Major	Minor
Cefoperazone[b]									
2 g i.v. q12h	35–75	≤16	>64	75	≥21	≤14	0.24	0.00	8.08
2 g i.v. q12h	25–42	≤32	>64	75	≥18	≤14	0.95	0.23	5.46
2 g i.v. q12h	35–75	≤16	>32	30	≥18	≤14	0.71	0.48	9.26
1 g i.v. q12h	25–45	≤16	>32	30	≥18	≤14	0.71	0.48	9.26
1 g i.v. q12h	40–50	≤8.0	>16	30	≥21	≤17	0.00	1.19	11.16
Cefotaxime[c]									
2 g i.v. q8h	30–45	≤8.0	>32	30	≥23	≤14	0.50	0.00	ND[d]
Cefamandole[e]									
2 g i.v. q8h	35–45	≤8.0	>16	30	≥18	≤14	1.50	0.30	5.30

[a] In vivo pharmacology is expressed as the percentage of dosage interval (8 or 12 h intravenously [i.v.]) that the serum drug concentration was at or above the in vitro correlative susceptible concentration. The efficacy of the in vitro tests is tabulated in percent error for very major (false-susceptible), major (false-resistant), and minor categories (one in vitro result was indeterminant).

[b] The cefoperazone human pharmacology was derived from references 2, 5, 18, and 28, with the in vitro susceptibility data from the current study. q12h, Every 12 h.

[c] Cefotaxime human pharmacology is as reported by Lode et al. in a comparative trial with cefoperazone (18), and the in vitro susceptibility results are taken from data presented by Fuchs and colleagues (8). q8h, Every 8 h.

[d] ND, Not determined.

[e] Cefamandole comparative human pharmacology was from Craig in a comparative study with cefoperazone, and the in vitro susceptibility results were taken from Jones et al. (a study of the cefuroxime 30-μg disk; in press).

ble) range (3, 8). Therefore, a need for a wide indeterminate zone range is minimized. The 3-mm indeterminate range contributes only a small number of minor errors and is equivalent to one $\log_2$ dilution interval. Preliminary interpretive criteria for the ICS disk diffusion method, using IsoSensitest (Oxoid), DST, or Mueller-Hinton agar, are very similar to those presented here (H. Grimm, personal communication). The latter recommended a ≤16-μg/ml susceptible breakpoint correlating with zones of ≥16 or ≥17 mm; a broader indeterminate category was also applied. The ICS method's interpretive criteria were derived strictly from regression lines (0.03 to 256 μg/ml) and without error rate considerations that may result in the slightly larger zone size breakpoints presented here.

There is currently a substantial amount of cefoperazone quality control data (Table 2), and the available statistical interpretation also seems to be valid. Even though the zones of inhibition reported in the controlled series and the random clinical series were not normally distributed, the applied statistical methods (Gavan's median technique and traditional mean ± 2 standard deviations) resulted in very similar quality control ranges. A final selection of ranges awaits Food and Drug Administration and NCCLS rulings.

Cross-resistance studies of 69 *P. aeruginosa* strains revealed an incomplete predictibility between those cephalosporins active against this species. Beta-lactamase hydrolysis studies demonstrated cefoperazone and cefsulodin to be most labile to the beta-lactamases produced by some strains of *P. aeruginosa*. Ceftazidime was most stable, yet two strains had ceftazidime MICs of ≥32 μg/ml, probably due to decreased drug access to cell target sites. The frequency of occurrence of these resistances will depend upon the endemic *P. aeruginosa* populations in any geographic area or hospital. An analysis of a large sample of *P. aeruginosa* strains from different geographic areas showed only 7 and 4.2% resistance to cefoperazone and ceftazidime, respectively (12; Jones et al., in press). The use of the cefoperazone MIC or disk diffusion test result to predict cefsulodin or ceftazidime susceptibility might be considered since significant false susceptibility results were not produced. The few cefoperazone-resistant *P. aeruginosa* strains could then be tested by a specific cefsulodin or ceftazidime test if treatment with these agents were still being considered. Only ceftazidime has a usable spectrum against *Pseudomonas* spp. other than *P. aeruginosa* (16, 24, 31, 32).

We believe that the beta-lactamases produced

by mutant subpopulations of *Enterobacter* will not adversely affect cefoperazone and cause false susceptibility by the various test methods (7). Using NCCLS reference broth procedures, no discrepant results were found between the MIC (resistant) and the disk zone (susceptible) commonly seen with cefamandole. Several investigators have noted susceptible cefoperazone agar dilution MICs with TEM plasmid-containing bacteria that readily hydrolyze the drug (unpublished data; F. Kayser personal communications). A broth dilution MIC with these same strains produces cefoperazone MICs of ≥64 μg/ml or resistant. Challenge *Enterobacteriaceae* strains known to produce type III-TEM beta-lactamases were used to establish these interpretive criteria. All of the isolates (10 from two species) were resistant by disk diffusion and broth dilution tests.

In conclusion, the cefoperazone disk test interpretive criteria with the 30- and 75-μg disks seem to be well established. Recommendations for use of each disk content are identical (≥18 mm = susceptible and ≤14 mm = resistant), but the correlate MICs differ by one $\log_2$ dilution step. We prefer to use the 75-μg disk if dosages of 2 g/12 h are used routinely or 30-μg disks if lower dosages are used. In either case, enterococci should not be tested, thus minimizing very major and other errors to <5%. Also, quality control performance ranges have been determined in structured interlaboratory trials and by in-use data from clinical investigators. Lastly, the cross-resistance analyses presented earlier (29) and in this paper favor the use of a separate cefoperazone disk for testing *Enterobacteriaceae*, gram-positive cocci, and *P. aeruginosa*. The cefoperazone susceptibility results against *P. aeruginosa* may be used for cefsulodin and ceftazidime susceptibility. Of the three antipseudomonas drugs, cefoperazone was least active, and thus it is the most appropriate representative for in vitro testing (i.e. minimal number of false-susceptible results produced). Cefoperazone should be a welcome therapeutic addition for treatment of a wide variety of serious infections, and the in vitro tests of its susceptibility appear to be ready for clinical laboratory application.

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ADDENDUM IN PROOF

Since this paper was accepted for publication, the NCCLS Disk Diffusion Test Subcommittee has selected the following interpretative criteria: susceptible, ≥21 mm (≤16 μg/ml); and resistant, ≤14 mm (>64 μg/ml). The quality control limits for the daily controls with the 75-μg cefoperazone disk are as follows: *E. coli* ATCC 25922, 28 to 34 mm; *S. aureus* ATCC 25923, 24 to 33 mm; and *P. aeruginosa* ATCC 27853, 23 to 29 mm. See NCCLS M2-A2-S2 for details.

LITERATURE CITED

1. **Baker, C. N., C. Thornsberry, and R. N. Jones.** 1980. In vitro antimicrobial activity of cefoperazone, cefotaxime, moxalactam (LY127935), azlocillin, mezlocillin, and other beta-lactam antibiotics against *Neisseria gonorrhoeae* and *Haemophilus influenzae*, including beta-lactamase-producing strains. Antimicrob. Agents Chemother. **17**:757–761.
2. **Balant, L., P. Dayer, M. Rudhardt, A. F. Allaz, and J. Fabre.** 1980. Cefoperazone: pharmacokinetics in humans with normal and impaired renal function and pharmacokinetics in rats. Clin. Ther. **3**:50–59.
3. **Barry, A. L., C. Thornsberry, R. N. Jones, and E. H. Gerlach.** 1980. Tentative interpretive standards for disk susceptibility tests with moxalactam (LY127935). Antimicrob. Agents Chemother. **18**:716–721.
4. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. **45**:493–496.
5. **Craig, W. A.** 1980. Single dose pharmacokinetics of cefoperazone following intravenous administration. Clin. Ther. **3**:46–49.
6. **Curtis, N. A. C., D. Orr, G. W. Ross, and M. G. Boulton.** 1979. Affinities of penicillins and cephalosporins for the penicillin-binding proteins of *Escherichia coli* K-12 and their antibacterial activity. Antimicrob. Agents Chemother. **16**:533–539.
7. **Findell, C. M., and J. C. Sherris.** 1976. Susceptibility of *Enterobacter* to cefamandole: evidence for a high mutation rate to resistance. Antimicrob. Agents Chemother. **9**:970–974.
8. **Fuchs, P. C., A. L. Barry, C. Thornsberry, R. N. Jones, T. L. Gavan, E. H. Gerlach, and H. M. Sommers.** 1980. Cefotaxime: in vitro activity and tentative interpretive standards for disk susceptibility testing. Antimicrob. Agents Chemother. **18**:88–93.
9. **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.
10. **Heymes, R., A. Lutz, and E. Schrinner.** 1977. Experimental evaluation of HR 756, a new cephalosporin derivative: pre-clinical study. Infection **5**:259–260.
11. **Jones, R. N., A. L. Barry, C. Thornsberry, and H. W. Wilson.** 1981. In vitro antimicrobial activity evaluation of cefodizime (HR221), a new semisynthetic cephalosporin. Antimicrob. Agents Chemother. **20**:760–768.
12. **Jones, R. N., P. C. Fuchs, A. L. Barry, T. L. Gavan, H. M. Sommers, and E. H. Gerlach.** 1980. Cefoperazone (T-1551), a new semisynthetic cephalosporin: comparison with cephalothin and gentamicin. Antimicrob. Agents Chemother. **17**:743–749.
13. **Jones, R. N., P. C. Fuchs, H. M. Sommers, T. L. Gavan, A. L. Barry, and E. H. Gerlach.** 1980. Moxalactam (LY127935), a new semisynthetic 1-oxa-beta-lactam antibiotic with remarkable antimicrobial activity: in vitro comparison with cefamandole and tobramycin. Antimicrob. Agents Chemother. **17**:750–756.
14. **Kaminura, T., Y. Matsumoto, N. Okada, Y. Mine, M. Nishida, S. Goto, and S. Kuwahara.** 1979. Ceftizoxime (FK 749), a new parenteral cephalosporin: in vitro and in vivo antibacterial activities. Antimicrob. Agents Chemother. **16**:540–548.
15. **Kayser, F. A.** 1980. Microbiological studies of cefoperazone. Clin. Ther. **3**:24–33.

16. **King, A., K. Shannon, and I. Phillips.** 1980. In vitro antibacterial activity and susceptibility of cefsulodin, an antipseudomonal cephalosporin, to beta-lactamase. Antimicrob. Agents Chemother. **17**:165–169.
17. **Kojo, H., M. Nishida, S. Goto, and S. Kuwahara.** 1979. Antibacterial activity of ceftizoxime (FK 749), a new cephalosporin, against cephalosporin-resistant bacteria and its stability to beta-lactamase. Antimicrob. Agents Chemother. **16**:549–553.
18. **Lode, H., B. Kemmerich, P. Koeppe, D. Belmega, and H. Jendoschek.** 1980. Comparative pharmacokinetics of cefoperazone and cefotaxime. Clin. Ther. **3**:80–88.
19. **Metzler, C. M., and R. M. DeHaan.** 1974. Susceptibility tests of anaerobic bacteria: statistical and clinical considerations. J. Infect. Dis. **130**:588–594.
20. **National Committee for Clinical Laboratory Standards.** 1979, 1981. Approved standard M2-A2S. Performance standards for antimicrobic disc susceptibility tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.
21. **National Committee for Clinical Laboratory Standards.** 1980. Proposed standard M7-P. Standard methods for dilution antimicrobial susceptibility tests for bacteria which grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
22. **Neu, H. C., N. Aswapokee, K. P. Fu, and P. Aswapokee.** 1979. Antibacterial activity of a new 1-oxa cephalosporin compared with that of other lactam compounds. Antimicrob. Agents Chemother. **16**:141–149.
23. **Neu, H. C., K. P. Fu, N. Aswapokee, P. Aswapokee, and K. Kung.** 1979. Comparative activity and beta-lactamase stability of cefoperazone, a piperazine cephalosporin. Antimicrob. Agents Chemother. **16**:150–157.
24. **O'Callaghan, C. H., P. Acred, P. B. Harper, D. M. Ryan, S. M. Kirby, and S. M. Harding.** 1980. GR-20263, a new broad-spectrum cephalosporin with antipseudomonal activity. Antimicrob. Agents Chemother. **17**:876–883.
25. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1971. Novel method for detection of beta-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:283–288.
26. **Richmond, M. H., and R. B. Sykes.** 1973. The beta-lactamases of gram-negative bacteria and their possible physiological role. Adv. Microb. Physiol. **9**:31–88.
27. **Schindler, P., and G. Huber.** 1980. Use of PADAC, a novel chromogenic beta-lactamase substrate, for the detection of beta-lactamase producing organisms and assay of beta-lactamase inhibitors/inactivators, p. 169–176. *In* U. Brodbeck (ed.), Enzyme inhibitors. Verlag Chemie, Weinheim.
28. **Shimizu, K.** 1980. Cefoperazone: absorption, excretion, distribution and metabolism. Clin. Ther. **3**:60–79.
29. **Thornsberry, C., A. L. Barry, R. N. Jones, C. N. Baker, and R. E. Badal.** 1982. Tentative interpretive standards for agar disk diffusion antimicrobial susceptibility testing of cefoperazone. J. Clin. Microbiol. **15**:769–776.
30. **Tsuchiya, K., M. Kondo, M. Kida, M. Nakao, T. Iwahi, T. Nishi, Y. Noji, M. Takeuchi, and Y. Nozaki.** 1981. Cefmenoxime (SCE-1365), a novel broad-spectrum cephalosporin: in vitro and in vivo antibacterial activities. Antimicrob. Agents Chemother. **19**:56–65.
31. **Tsuchiya, K., M. Kondo, and H. Nagatoma.** 1978. SCE-129, antipseudomonal cephalosporin: in vitro and in vivo antibacterial activities. Antimicrob. Agents Chemother. **13**:137–145.
32. **Verbist, L., and J. Verhaegen.** 1980. GR-20263: a new aminothiazolyl cephalosporin with high activity against *Pseudomonas* and *Enterobacteriaceae*. Antimicrob. Agents Chemother. **17**:807–812.
33. **Welch, D. F., B. J. Saxon, D. N. Wright, and J. M. Matsen.** 1980. In vitro activity and laboratory testing considerations of cefoperazone (T-1551), p. 176–179. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious diseases. American Society for Microbiology, Washington, D.C.