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Cefotaxime: In Vitro Activity and Tentative Interpretive Standards for Disk Susceptibility Testing

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Tested against 9,412 recent clinical isolates, cefotaxime exhibited 8 to 64 times greater activity against the *Enterobacteriaceae* than did cephalothin and two to four times greater activity against *Pseudomonas aeruginosa*, but only one-half to one-eighth the activity of cephalothin against staphylococci. Using 420 different clinical isolates, but with comparable minimal inhibitory concentration (MIC) distributions, disk diffusion-MIC regression analyses were performed, using 5- and 30-μg cefotaxime disks. Cefotaxime MIC susceptible and resistant breakpoints of ≤8 and >32 μg/ml are tentatively proposed. Based on the MIC breakpoints, the data showed the best discrimination among the three susceptibility categories (susceptible, indeterminate, and resistant) when the 30-μg cefotaxime disk was used. The zone diameter breakpoints as determined by the error rate-bounded method and regression analysis were ≥23 mm for susceptible, 15 to 22 mm for indeterminate, and ≤14 mm for resistant.

Cefotaxime (formerly HR 756) is a new cephalosporin agent much acclaimed for its broad spectrum of in vitro antimicrobial activity (2, 3, 5, 11-13) and its beta-lactamase resistance and beta-lactamase inhibitory activity (5, 6, 8). Interpretive zone standards for the disk diffusion susceptibility testing of cefotaxime have been recently proposed (1) and include the use of 5- or 30-μg disks, depending on the organism being tested. This report summarizes our disk diffusion susceptibility data on cefotaxime and proposes somewhat different tentative interpretive zone standards, based on the distribution of minimal inhibitory concentrations (MICs) among the various pathogens whose infections are likely to be treated with cefotaxime, achievable serum levels of the drug, and statistical considerations.

MATERIALS AND METHODS

Cefotaxime was supplied by Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J. Cephalothin laboratory-standard powder was provided by Eli Lilly Research Laboratories, Indianapolis, Ind. Both compounds were diluted in Mueller-Hinton broth supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) or included in Mueller-Hinton agar plates, as previously described in detail (6, 7).

In the first portion of this study, the clinical isolate MIC study, the 9,412 organisms tested were consecutive clinical strains isolated by six participating laboratories during a 45-day period as described previously (6, 7). A twofold dilution protocol was used for each antibiotic, ranging from 0.5 to 32 μg/ml. Daily, each participating laboratory tested quality control organisms which included *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). The results of these fell within the mode ± 1 dilution in more than 97% of tests.

The second portion of the study was a regression analysis of 420 clinical bacterial isolates obtained from six of the collaborating laboratories. These included 85 strains of *P. aeruginosa*, 28 *Pseudomonas* species, 15 *Acinetobacter calcoaceticus* var. *anitratus*, 25 *Escherichia coli*, 50 *Enterobacter* species, 25 *Klebsiella pneumoniae*, 25 *Serratia* species, 20 *Citrobacter* species, 10 *Salmonella* species, 25 *Proteus mirabilis*, 55 indole-positive *Proteus* and *Providencia* species, 49 *Staphylococcus aureus*, and 8 *Streptococcus faecalis*. In this phase, the twofold dilution sequence included cefotaxime and cephalothin concentrations of 0.125 to 64 μg/ml. Cefotaxime 30- and 5 μg disks and cephalothin 30-μg disks were prepared by Difco Laboratories, Detroit, Mich. These disks had a mean assay of 110.5% of stated potency by the disk-plate procedure. The disk diffusion susceptibility tests were performed, according to the latest procedure published by the National Committee for Clinical Laboratory Standards (NCCLS) (10), by two participating laboratories [Center for Disease Control, Atlanta, Ga., and University of California (Davis) Medical Center, Sacramento, Calif.]. Diffusion zone diameters and MICs for each

organism were plotted as scattergrams. Regression analyses were made by the formula of least squares as adapted for computer computation. In addition, analyses were performed by the error rate-bounded method of Metzler and DeHaan (9). Cross-resistance analyses comparing cefotaxime, cephalothin, cefamandole, and cefoxitin were also performed, using the 420 strains listed above.

RESULTS

The susceptibility of 9,412 clinical aerobic and facultatively anerobic isolates to cefotaxime in this study was high, and the results shown in Table 1 and Fig. 1 and 2 generally agree with previously published reports (2, 5, 11, 13). Each major organism group had a characteristic MIC distribution, often with little overlap (Fig. 2). Over 91% of *Enterobacteriaceae* were inhibited by ≤0.5 μg of cefotaxime per ml, which was 8 to 64 times greater than the activity of cephalothin.

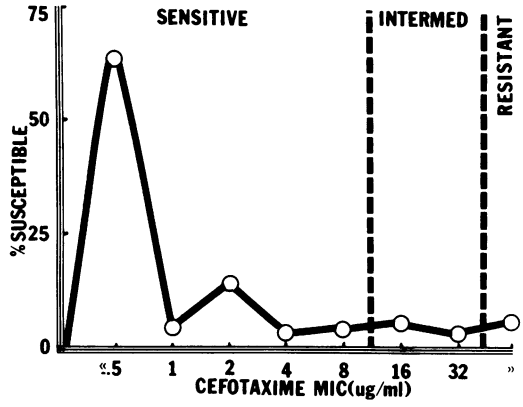

FIG. 1. *Cefotaxime (HR 756) MIC population distribution for 9,412 recent clinical isolates. Eight-six percent of strains are found in the susceptible category.*

TABLE 1. *MIC-50 and MIC-90 of cefotaxime and cephalothin against 9,412 clinical isolates from six laboratories*[a]

Organism	No. of isolates	Drug[b]	MIC-50 (mg/ml)	MIC-90 (mg/ml)
Escherichia coli	2,813	Ctax	≤0.5	≤0.5
		Ceph	4.0	32.0
Klebsiella	1,182	Ctax	≤0.5	≤0.5
		Ceph	4.0	16.0
Enterobacter	715	Ctax	≤0.5	8.0
		Ceph	>32	>32
Serratia	264	Ctax	≤0.5	4.0
		Ceph	>32	>32
Citrobacter	235	Ctax	≤0.5	≤0.5
		Ceph	32	>32
Proteus mirabilis	487	Ctax	≤0.5	≤0.5
		Ceph	4.0	8.0
Proteus (indole positive)	221	Ctax	≤0.5	1.0
		Ceph	>32	>32
Miscellaneous *Enterobacteriaceae*	166	Ctax	≤0.5	≤0.5
		Ceph	4.0	>32
Pseudomonas aeruginosa	825	Ctax	16.0	>32
		Ceph	>32	>32
Acinetobacter anitratus	132	Ctax	8.0	32
		Ceph	>32	>32
Miscellaneous (gram-negative bacilli)	146	Ctax	8.0	>32
		Ceph	>32	>32
Staphylococcus aureus	1,219	Ctax	2.0	2.0
		Ceph	≤0.5	1.0
S. epidermidis	392	Ctax	1.0	8.0
		Ceph	≤0.5	1.0
Enterococci	453	Ctax	>32	>32
		Ceph	32	32
Miscellaneous (gram-positive bacteria)	162	Ctax	≤0.5	≤0.5
		Ceph	≤0.5	2.0
Total All isolates	9,412	Ctax	≤0.5	16.0
		Ceph	8.0	>32

[a] MIC-50 and MIC-90, MICs necessary to inhibit 50 and 90%, respectively, of isolates.
[b] Ctax, Cefotaxime; Ceph, cephalothin.

Staphylococcus aureus, the second most susceptible major organism population to cefotaxime, required a mean of 2 μg of cefotaxime per ml for inhibition of growth, which was two to four times that required of cephalothin. The mean and modal MICs of cefotaxime for *A. calcoaceticus* var. *anitratus* and *Pseudomonas aeruginosa* were 8.0 and 16.0 μg/ml, respectively, and were significantly less than those of cephalothin (>32 μg/ml for each).

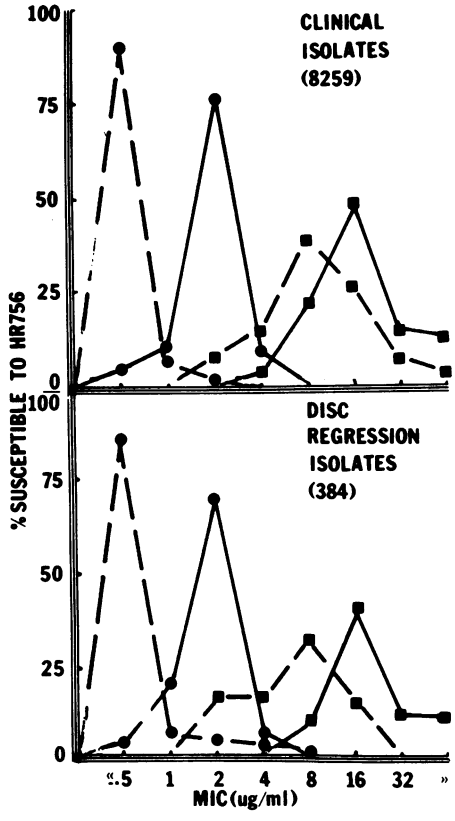

FIG. 2. *Cefotaxime (HR 756) MIC distributions of the clinical isolates and major organism groups used in the disk diffusion regression analysis belonging to the Enterobacteriaceae (●----●), S. Aureus (●——●), P. aeruginosa (■——■), and A. calcoaceticus var. anitratus (■----■).*

The 420 strains used in the regression analysis study had an MIC distribution for these major groups of organisms comparable to those found among current clinical isolates (Fig. 2).

Cross-resistance analysis of cefotaxime, cephalothin, cefamandole, and cefoxitin is summarized in Table 2. Cefotaxime was the most active cephalosporin against the 420 organisms tested, with 22 to 39% of strains being resistant to one or more of the comparable drugs, yet susceptible to cefotaxime.

The regression analysis of the 30-μg cephalothin disk yielded a slope of −0.317, a *y*-axis intercept of 726 μg/ml, and a correlation coefficient of 0.90. The corresponding figures for the 5- and 30-μg cefotaxime disks were −0.289, 119 μg/ml, and 0.94, and −0.323, 1,121 μg/ml, and 0.87, respectively. Figures 3 and 4 display the regression scattergrams for the 5- and 30-μg cefotaxime disks, respectively. By error-rate bounding, the susceptible zone size breakpoints for the 30-μg cephalothin and cefotaxime disks were ≥18 and ≥23 mm, respectively.

DISCUSSION

Available pharmacological data on cefotaxime suggest that selecting an MIC susceptible breakpoint of 8 μg/ml is reasonable. One gram of cefotaxime given intramuscularly produces peak serum levels of 20 μg/ml (1); 1 g given intravenously over 15 min produces a peak level of 93 μg/ml (8); and 1 g per h by intravenous infusion maintains a steady-state level of 64 μg/ml (8). Furthermore, this would place the two major clearly susceptible (in vitro) groups of organisms (*Enterobacteriaceae* and *S. aureus*) in the susceptible category and would also be consistent with the susceptible breakpoints of other cephalosporins. In contrast to the resistant MIC breakpoint for cephalothin, we have selected >32 μg/ml as the resistant MIC breakpoint for cefotaxime. This is based on two significant considerations. (i) Pharmacologically, cefotaxime

TABLE 2. *Cross-resistance analysis of 420 organisms used to develop disk diffusion susceptibility testing criteria*

MIC	≥64 μg/ml	≥16 μg/ml (resistant)			
	Cefotaxime	Cefotaxime	Cephalothin	Cefamandole	Cefoxitin
≤ 32 μ/ml					
Cefotaxime			239[a]	165	176
≤8 μg/ml (susceptible)					
Cefotaxime			165	91	102
Cephalothin	0	0		0	0
Cefamandole	0	0	73		35
Cefoxitin	0	0	62	24	

[a] Number of isolates resistant to the column antibiotic and susceptible to the row antibiotic at MIC breakpoints indicated.

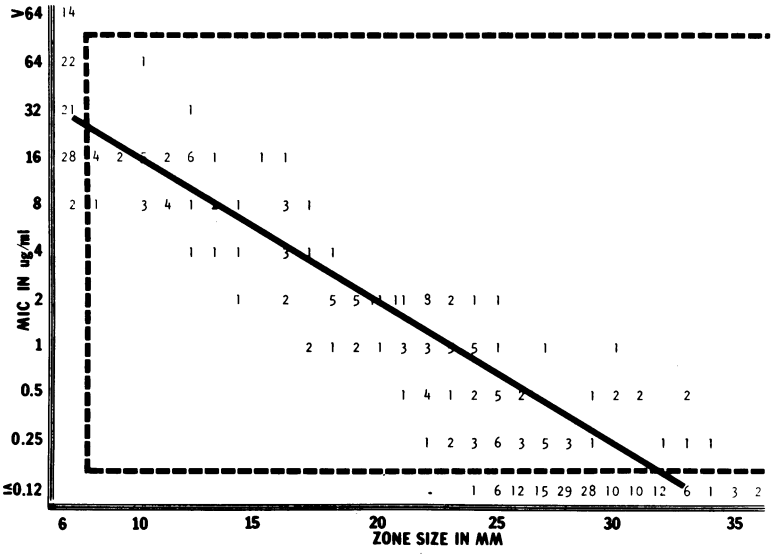

FIG. 3. *Correlation between MICs and zone diameters, using 5-µg cefotaxime disks. Values outside the broken line were excluded from the regression analysis. (Slope = −0.289, y-axis intercept = 119 µg/ml, correlation coefficient = 0.94.)*

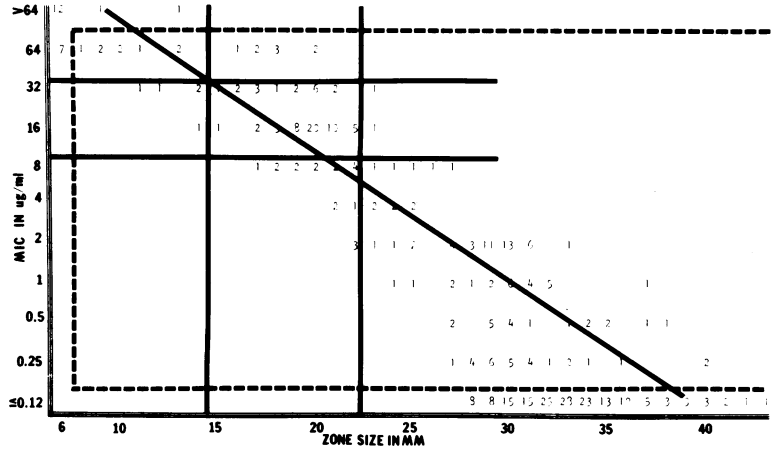

FIG. 4. *Correlation between MICs and zone diameter, using 30-µg cefotaxime disks. Values outside the broken line were excluded from the regression analysis. Horizontal lines represent proposed MIC resistant (upper line) and susceptible (lower line) breakpoints. Vertical lines represent the proposed zone size susceptible (right line) and resistant (left line) breakpoints as determined by the error rate-bounded method (9). (Slope = −0.323, y-axis intercept = 1,121 µg/ml, correlation coefficient = 0.87.)*

levels well above this are achievable in the serum; e.g., 2 g given intravenously over 15 min produces peak levels of 160 µg/ml (3). (ii) The majority of *P. aeruginosa* isolates would then fall into the indeterminate category of 16 to 32 µg/ml. Since it is not yet known how *P. aeruginosa* infections will respond in vivo to cefotaxime, it may be best to refer this category tentatively as indeterminate rather than intermediate.

It is now well recognized that the class disk concept is no longer valid for all cephalosporins. Of the 420 isolates used for the disk diffusion analysis, all strains that were resistant to ≥16 µg of cefotaxime per ml were also resistant to cephalothin, cefamandole, and cefoxitin. However, 29% of these strains were resistant to ≥16 µg of cephalothin per ml, 22% were resistant to ≥16 µg of cefamandole per ml, and 24% were resistant to ≥16 µg of cefoxitin per ml while

being susceptible to ≤8 μg of cefotaxime per ml. Therefore, the disks of neither cephalothin nor the two second-generation drugs would be appropriate for predicting cefotaxime susceptibility.

Examination of the cefotaxime regression plot for the 5-μg disk (Fig. 3) shows that 68% of strains inhibited by 16 and 32 μg/ml gave no zones of inhibition around the 5-μg disk, rendering this disk essentially useless for determining the indeterminate category. On the other hand, all of these strains had zones of inhibition around the 30-μg disk (Fig. 4). Aswapokee et al. (1) suggested the use of both 5- and 30-μg disks for different genera. This would require either prior identification of the organism being tested or the simultaneous use of both 5- and 30-μg disks, both of which are impractical. The 30-μg disk alone, however, quite clearly differentiated the three major categories according to MIC breakpoints as follows: susceptible, ≤8 μg/ml; indeterminate, 16 to 32 μg/ml; and resistant, >32 μg/ml. With ≤8 μg/ml as the susceptible MIC breakpoint, error-rate bounded analysis of the 30-μg cefotaxime disk data (with acceptability of ≤1% false-susceptible) yielded a susceptible zone size breakpoint (Zs) of ≥23 mm. The actual false-susceptibility rate at this Zs was 0.5%. With ≤5% as an acceptable false-resistance rate, the resistant zone size breakpoint (Zr) would also be 23 mm (assuming no indeterminate zone); i.e., Zr would equal Zs. For reasons stated above, an indeterminate zone was included; based on regression analysis, the Zr was 14 mm. Application of this Zr results in a reduction of the false-resistance rate from 4.5% (using Zr = 23 mm) to 0%. Despite the rather broad indeterminate range (15 to 22 mm), only 7% of the 9,412 clinical isolates fell into this category, of which 69% were *P. aeruginosa*. Susceptibility by the disk test (zone ≥ 23 mm) also correlates with an MIC breakpoint of ≤4 μg/ml; most (13 of 18) *Pseudomonas* and *Acinetobacter* species inhibited by 8 μg/ml gave indeterminate zones of 15 to 22 mm.

We disagree with Aswapokee et al. (1), who state that it is necessary to have different susceptibility criteria for *Enterobacteriaceae* (6 μg/ml) versus *P. aeruginosa* (25 μg/ml) for cefotaxime. Such breakpoints may be representative of the MIC distributions of these two groups of organisms (e.g., most *Enterobacteriaceae* are susceptible to less than 6 μg of cefotaxime per ml). But the use of two different interpretive criteria for the susceptibility of two groups of bacteria implies either (i) that there are different recommended doses for the treatment of each group (which is not the case to date) or (ii) that the clinical responses of these two groups of microorganisms to cefotaxime are different. Although few *Enterobacteriaceae* require more than 6 μg of cefotaxime per ml for inhibition, there is no evidence yet to suggest that an *Escherichia coli* strain, for example, whose cefotaxime MIC is 25 μg/ml will respond any differently than a *P. aeruginosa* strain with the same MIC. Therefore, there is no reason at this time to propose two separate interpretive criteria. The more meaningful and significant consideration is the differentiation between organisms of any species whose cefotaxime MIC is greater than or less than 25 μg/ml. The use of the 30-μg disk alone clearly accomplishes this.

Although the cefotaxime regression lines calculated and drawn on Fig. 3 and 4 are straight lines, it is apparent by examining the scattergram that the actual distribution tends to be parabolic at the more susceptible end of the curve. This is more striking with the 30-μg cefotaxime disk than with the 5-μg disk. If the susceptible MIC breakpoint were lower (e.g., 1 μg/ml), then the 30-μg disk would be unable to distinguish between the susceptible and indeterminate categories. In fact, in the region of the two MIC breakpoints selected, the regression line with the 30-μg disk is straight and permits the best categorization by the disk diffusion test. The inability to differentiate MICs of 2 μg/ml from those of 0.25 μg/ml is inconsequential since both are clearly in the susceptible category, an intrinsic limitation of the disk diffusion test.

In summary, the tentative interpretive standards for cefotaxime disk susceptibility testing by the NCCLS procedure (10) that we propose are: disk content, 30 μg; susceptible zone size, ≥23 mm; indeterminate zone size, 15 to 22 mm; resistant zone size, ≤14 mm. The advantages of these are as follows. (i) A single disk is used for all rapidly growing facultative and aerobic bacteria, which clearly delineates the three standard categories of susceptibility expected from disk diffusion tests. (ii) With the exception of *A. calcoaceticus* var. *anitratus*, the major portion of common clinical species is distributed in single susceptibility categories: *Enterobacteriaceae* and *S. aureus*, susceptible; *P. aeruginosa*, indeterminate; and enterococci, resistant. The major objection to the use of the 30-μg disk mentioned previously (1) is that the zone sizes of many *Enterobacteriaceae* would be so large that they would interfere with the testing of adjacent antibiotics. Yet the data presented in this report (1) showed only a 3-mm difference in maximum zone size for the two disks (35 mm for 5-μg disk versus 38 mm for 30-μg disk). Our data showed a difference of only 2 to 3 mm between the mean zone diameter of the two disks at MICs of ≤0.5 μg/ml. Only 8% of strains tested gave zone sizes

greater than 35 mm with the 30-μg disk. We do not believe that this poses a significant enough problem to warrant a two-disk standard for this agent, and that the problem can be largely abrogated by judicious positioning of antibiotic disks on the agar plate.

It should be emphasized that these proposed standards should be considered tentative. The three major considerations in establishing interpretive standards for disk susceptibility have been discussed in detail elsewhere (A. Barry et al., submitted for publication). Two of these factors (MIC distribution of clinical isolates likely to be treated, and achievable drug serum levels) have been used in establishing the above proposed standards. The third major factor, clinical experience in the therapy of different infections by different microbes, was not used because such data are not yet available. It is this deficiency that makes these proposed criteria tentative. The proposed susceptible breakpoint at this time appears to be reliable and firm. The breakpoint for resistance is the one most in question and must await the outcome of clinical trials. Although cefotaxime serum levels of 16 and 32 μg/ml are readily exceeded, the majority of clinical isolates requiring these levels for growth inhibition in vitro are *P. aeruginosa* and other gram-negative, nonfermenting bacteria. Until clinical studies confirm or refute the efficacy of cefotaxime in infections by such microbes, we consider MICs and zone sizes in the intermediate zone as indeterminate.

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