

## Diffusion Disk Susceptibility Testing with Cefotaxime

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The diffusion disk susceptibility of *Staphylococcus aureus*, *Enterobacteriaceae*, and *Pseudomonas aeruginosa* to cefotaxime was determined. A 30- $\mu$ g disk provided data for the susceptibility of *P. aeruginosa*, but yielded extremely large zones of inhibition against *Enterobacteriaceae*. A 5- $\mu$ g disk seemed to provide the most useful susceptibility data for *S. aureus* and *Enterobacteriaceae*.

Cefotaxime is a novel cephalosporin compound which has been shown to have a broad spectrum of antibacterial activity that includes most gram-positive and -negative aerobic and anaerobic bacteria (2-4, 6, 7). The agent is resistant to hydrolysis by most  $\beta$ -lactamases and also has the ability to inhibit many bacteria resistant to other  $\beta$ -lactam agents by virtue of permeability factors (3). Since the current cephalosporin class disk, cephalothin, will not predict susceptibility to cefotaxime, we wished to determine the optimal disk content and characteristics to use for susceptibility testing.

### MATERIALS AND METHODS

A total of 193 bacterial isolates were studied. Organisms were recent clinical isolates from blood, sputum, urine, wounds, and stools from patients hospitalized at Columbia-Presbyterian Medical Center, New York, N.Y. These included *Staphylococcus aureus* (23 isolates), *Escherichia coli* (12 isolates), *Enterobacter* (15 isolates), *Klebsiella pneumoniae* (11 isolates), indole-positive *Proteus* (15 isolates), *Proteus mirabilis* (12 isolates), *Citrobacter* (10 isolates), *Acinetobacter* (6 isolates), *Salmonella* (5 isolates), *Shigella* (6 isolates), *Serratia marcescens* (5 isolates), and *Pseudomonas aeruginosa* (73 isolates). In each species organisms resistant to ampicillin, carbenicillin, and cephalothin were used. Control organisms used in both agar dilution and diffusion disk susceptibility were *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Minimal inhibitory concentrations (MICs) for cefotaxime were determined by the agar dilution method (1). The antibiotic was serially diluted in sterile distilled water, with the concentrations 400 to 0.006  $\mu$ g/ml, and incorporated into Mueller-Hinton agar (Baltimore Biological Laboratory). A control plate without antibiotic was used in each series. Plates were inoculated with  $10^5$  organisms with a replicating device. Plates were then incubated at 35°C for 18 h. The MICs were defined as the lowest concentration of antibiotic which inhibited visible growth.

Disk diffusion studies were performed by a modification of a standard method described by the National Committee for Clinical Laboratory Standards (5).

Overnight cultures were diluted with Mueller-Hinton broth and adjusted to obtain a turbidity visually comparable to a 0.5-MacFarland standard opacity. The adjusted suspensions were then inoculated to give confluent growth onto plates (15 by 100 mm) containing 4-mm-deep Mueller-Hinton agar (Baltimore Biological Laboratory). Antibiotic susceptibility disks of standard size were prepared containing 2, 5, 10, and 30  $\mu$ g of cefotaxime. The disks were prepared at room temperature and kept frozen until use. Four disks of different concentrations were placed on the agar surface of a single plate. Zone sizes were measured with calipers after 18 h of incubation at 35°C. Agar dilutions and disk susceptibility were performed on the same day.

Control *E. coli* ATCC 25922 was used in every series of both agar dilution and disk diffusion. Zones sizes, in millimeters, were plotted on the abscissa against log<sub>2</sub> MICs on the ordinate, and the linear regression lines were obtained by computer calculation.

### RESULTS

It proved necessary to divide organisms into one group of *Enterobacteriaceae* and *S. aureus* and another group of *P. aeruginosa*. Figure 1 is a plot of the agar MICs of 119 *S. aureus* and *Enterobacteriaceae* with the zone sizes achieved when a 5- $\mu$ g test disk was used. Zone sizes ranged from no zone to 35 mm, and MICs ranged from 0.01 to 100  $\mu$ g/ml. There was a good correlation between MICs and zone sizes (Table 1), with only moderate scatter around the regression line. The regression line intersected the ordinate (the 6-mm or "no-zone" point) at a cefotaxime MIC of 85  $\mu$ g/ml. With the 2- $\mu$ g cefotaxime disk, there was good correlation of the zones of inhibition about the regression line (Table 1), but the no-zone point was 32  $\mu$ g/ml. In the case of the 10- $\mu$ g disk, the no-zone point was 1,200  $\mu$ g/ml and greater than 1,600  $\mu$ g/ml for the 30- $\mu$ g/disk (Fig. 2).

Most *Enterobacteriaceae*, *E. coli*, *Klebsiella*, *Enterobacter*, and *Proteus* were inhibited by less than 0.2  $\mu$ g of cefotaxime per ml, as they

have been in other studies (2, 4, 6, 7). The zones of inhibition for *Enterobacteriaceae* with the 10- $\mu$ g disk were 30 to 36 mm and 32 to 38 mm with the 30- $\mu$ g disk. With a zone of inhibition of 15 mm or greater as susceptible, organisms with MICs of 6.2  $\mu$ g/ml or less would be calculated as susceptible to cefotaxime with a 5- $\mu$ g disk.

The cefotaxime MICs of *P. aeruginosa* are distinctly higher than those for other organisms (2, 6, 7). As Fig. 3 illustrates with a 30- $\mu$ g disk, adequate zone sizes can be obtained, and the regression line shows a good correlation (Table 1) with the no-zone point at 100  $\mu$ g/ml. In contrast, with a 10- $\mu$ g disk the no-zone point is 45  $\mu$ g/ml and the zones of inhibition for 25  $\mu$ g/ml ranged from 8 to 20 mm (Fig. 4). The majority of *Pseudomonas* isolates gave no zone of inhibition when tested with the 2- and 5- $\mu$ g disks. With a zone of inhibition for the 30- $\mu$ g disk of 15 mm or greater, organisms with MICs of 25  $\mu$ g/

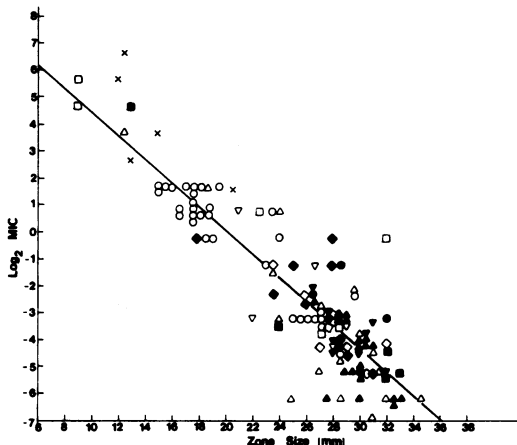


FIG. 1. Comparison of MICs of cefotaxime and the zone sizes obtained with a disk containing 5  $\mu$ g. Symbols:  $\circ$ , *S. aureus*;  $\bullet$ , *E. coli*;  $\diamond$ , *K. pneumoniae*;  $\blacklozenge$ , *Enterobacter*;  $\triangle$ , *Proteus*, indole-positive;  $\blacktriangle$ , *P. mirabilis*;  $\nabla$ , *S. sonnei*;  $\blacktriangledown$ , *Salmonella*;  $\square$ , *Acinetobacter*;  $\blacksquare$ , *Citrobacter*;  $\times$ , *S. marcescens*

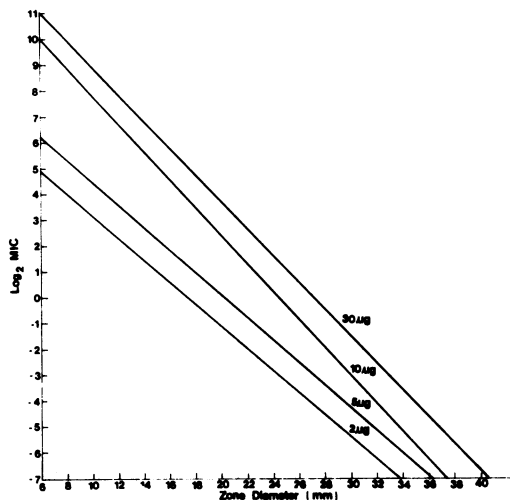


FIG. 2. Regression lines obtained for disks containing 2, 5, 10, and 30  $\mu$ g of cefotaxime tested against *Enterobacteriaceae* and *S. aureus*.

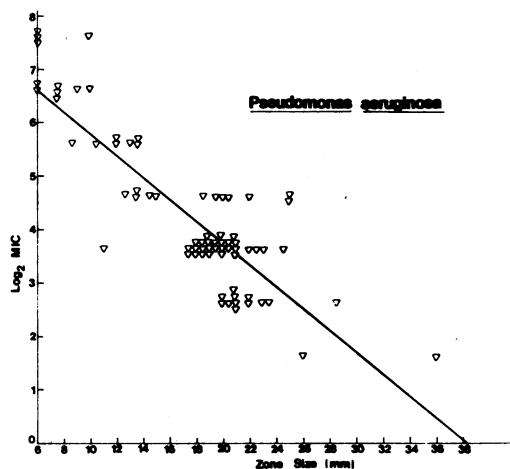


FIG. 3. Comparison of MICs of cefotaxime and zone sizes obtained with a 30- $\mu$ g disk of cefotaxime tested against 73 isolates of *P. aeruginosa*.

TABLE 1. Analysis of regression lines of data

Antibiotic disk concn ( $\mu$ g)	Organism (no.)	Correlation coefficient	Slope	y Intercept ( $\log_2$ MIC)	No zone ( $\mu$ g/ml)
2	<i>S. aureus</i> + <i>Enterobacteriaceae</i> (120)	0.9016	-0.427	17.38	32
5	<i>S. aureus</i> + <i>Enterobacteriaceae</i> (120)	0.9005	-0.439	18.8	85
10	<i>S. aureus</i> + <i>Enterobacteriaceae</i> (120)	0.8850	-0.550	23.45	1,014
30	<i>S. aureus</i> + <i>Enterobacteriaceae</i> (120)	0.8260	-0.500	27.9	1,927
10	<i>Pseudomonas</i> (73)	0.7377	-0.2013	6.678	45
30	<i>Pseudomonas</i> (73)	0.82426	-0.2064	7.848	100

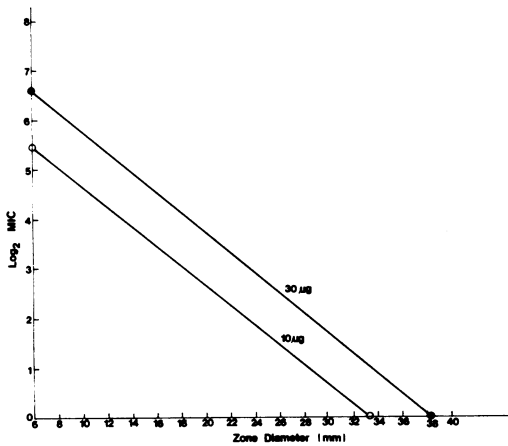


FIG. 4. Regression lines obtained for 10- and 30- $\mu$ g disks of cefotaxime tested against *P. aeruginosa*.

ml or less would be considered susceptible, and there was only one false-resistant organism.

#### DISCUSSION

It is clear that the class disk for cephalosporins, cephalothin, cannot be used to predict susceptibility to cefotaxime since cefotaxime inhibits most members of the *Enterobacteriaceae* and *P. aeruginosa* which are resistant to cephalothin. The extremely low cefotaxime MICs against *Enterobacteriaceae* and the higher MICs for *Pseudomonas* make selection of a disk of single concentration difficult. If only a low-concentration (2- or 5- $\mu$ g) disk is used, almost all *Pseudomonas* would not show a zone of inhibition. If a 30- $\mu$ g disk is used, the zones of inhibition for *E. coli*, *Klebsiella*, and *Enterobacter* would be so large that they would interfere with the testing of the adjacent antibiotics.

Studies in our laboratory (manuscript in preparation) have shown that peak serum levels of 11  $\mu$ g/ml are achieved after intramuscular administration of 500 mg and serum levels of 20  $\mu$ g/ml after a 1-g intramuscular injection. After intravenous infusion of 1 g, serum levels of 41  $\mu$ g/ml are achieved. It will be necessary to have different susceptibility criteria for *Enterobacteriaceae* and *S. aureus* from the susceptibility criteria for *P. aeruginosa*. We would set as susceptible 6  $\mu$ g/ml for the *Enterobacteriaceae* and *S. aureus* and 25  $\mu$ g/ml for *Pseudomonas*.

Using the 5- $\mu$ g cefotaxime disk to test susceptibility of *Enterobacteriaceae* and *S. aureus*, a

zone of inhibition of  $\geq 15$  mm would indicate susceptibility and a zone of  $\leq 13$  mm would indicate resistance. Using the 30- $\mu$ g disk to test susceptibility of *Pseudomonas* a zone of inhibition of  $\geq 15$  mm would indicate susceptibility, and a zone of  $\leq 13$  mm would indicate resistance. Only one isolate of either group tested would be considered falsely susceptible.

We did not examine the disk susceptibility of gram-positive species other than *S. aureus* since in most laboratories susceptibilities of *S. pneumoniae*, *S. pyogenes*, and *S. agalactiae* are not determined. Furthermore, these species are susceptible to cefotaxime (6). *S. faecalis* is resistant to cefotaxime in terms of achievable concentrations in serum (6). Enterococci with cefotaxime MICs of 6 to 25  $\mu$ g/ml had zones of 15 to 21 mm with the 5- $\mu$ g disk and of 18 to 26 mm with the 30- $\mu$ g disk. Cefotaxime has been shown to inhibit  $\beta$ -lactamase-producing *Haemophilus influenzae* and *Neisseria gonorrhoeae* (2, 6). The presence of a  $\beta$ -lactamase in these species is the usual mechanism of resistance to  $\beta$ -lactam compounds. Assays for the presence of a  $\beta$ -lactamase would be preferred to testing with a disk. The 30- $\mu$ g disk gave zones of inhibition for these organisms of 35 to 45 mm and the 5- $\mu$ g disk gave zones of 33 to 38 mm. The MICs of the organisms were 0.005 to 0.4  $\mu$ g/ml.

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