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In Vitro Studies with Cefazolin

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Susceptibilities of 259 isolates of pathogenic bacteria to cefazolin were measured by broth and agar dilution procedures. Beta-hemolytic streptococci were inhibited by 0.25 μg/ml, whereas *Staphylococcus aureus* and alpha-hemolytic streptococci were inhibited by 2.0 μg/ml. Enterococci were resistant to less than 32 μg/ml. Wide variation was seen with gram-negative species. Most isolates of *Klebsiella* species and *Proteus mirabilis* were inhibited by 4.0 or 8.0 μg/ml. *Escherichia coli* were less susceptible, and most isolates of *Pseudomonas aeruginosa*, *Serratia* species, and *Enterobacter* species were resistant to 128 μg/ml.

Cefazolin is a new cephalosporin C derivative which has been shown to be broad spectrum, bactericidal and resistant to penicillinase produced by *Staphylococcus aureus* (5). In vitro, it is equivalent in activity to cephalothin against *Diplococcus pneumoniae*, *S. aureus*, group A streptococci, and *Proteus mirabilis* and four to eight times more active against *Escherichia coli* (7). Pharmacological studies have shown that intramuscular doses of cefazolin are well tolerated (5) and that higher and more prolonged serum levels are obtainable as compared to those obtained with comparable doses of cephaloridine (2, 5, 7). It has been shown to be effective in the treatment of a variety of bacterial infections including surgical infections (9), bacterial pneumonias (11), bacterial endocarditis (6), and urinary tract infections (3).

The studies reported here were undertaken to provide additional data regarding the in vitro activity of cefazolin against several species of pathogenic bacteria and also to determine if there were significant differences in susceptibility data obtained with routine agar and broth dilution procedures.

Two hundred and fifty-nine recent clinical isolates of bacteria were tested. These included 25 isolates each of *Enterobacter* species, *E. coli*, *Klebsiella pneumoniae*, *P. mirabilis*, *Pseudomonas aeruginosa*, *Serratia* species, beta-hemolytic streptococci, enterococci, and *S. aureus*. Ten isolates of indole-positive *Proteus* species and 24 isolates of alpha-hemolytic streptococci also were tested. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as controls.

Minimal inhibitory concentrations (MICs) were determined in Mueller-Hinton broth (Difco) by a twofold dilution procedure (1). Concentrations of cefazolin ranged from 128 to 0.063 μg/ml. Inocula containing approximately 10^6 cells were prepared from 1:1,000 dilutions of overnight broth cultures. The MIC was defined as the lowest concentration of drug which inhibited growth as determined visually by the absence of turbidity after incubation at 37 C for 24 h. Minimal bactericidal concentrations (MBC) were determined by subculture, with a calibrated loop, of all negative tubes to drug-free media with subsequent incubation at 37 C for 24 h. The MBC was defined as the lowest concentration of drug which yielded less than five colonies on subculture. MICs also were termined using the International Collaborative Study agar dilution procedure (4) and Mueller-Hinton agar (BBL). Cefazolin was serially diluted in twofold increments so that final concentrations of drug would range from 128 to 0.063 μg/ml. Five percent sheep blood was added in tests with alpha- and beta-hemolytic streptococci and enterococci. Tests were performed with square, disposable petri dishes (100 by 15 mm). Test inocula prepared from overnight broth cultures, containing approximately 10^4 cells, were applied to the agar surfaces with an inoculating device similar to the Steer replicator (10). The MIC was defined as the lowest concentration of drug which inhibited colony formation after incubation at 37 C for 24 h. Sterile cefazolin standard (Eli Lilly and Co., lot 51-63-2B) was used in both studies.

Generally, there was good agreement between MIC values obtained with cefazolin in this study and those reported by previous investiga-

TABLE 1. *Inhibitory activity of cefazolin against 259 bacterial isolates as determined by the broth dilution procedure*[a]

Organism	No. tested	No. of isolates inhibited/cumulative % inhibited												
		0.063[b]	0.125	0.25	0.50	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128	>128
Gram-negative														
Enterobacter sp.	25							1/4					1/8	23/100
Escherichia coli	25						1/4	1/8	5/28	1/32	8/64	5/84	2/92	2/100
Klebsiella pneumoniae	25					1/4	5/24	10/64	2/72	3/84		1/88		3/100
Proteus mirabilis	25							7/28	11/72	6/96	1/100			
Proteus sp.[c]	10										1/10		2/30	7/100
Pseudomonas aeruginosa	25													25/100
Serratia sp.	25													25/100
Gram-positive														
Staphylococcus aureus	25			3/12	2/20	12/68	8/100							
Alpha-hemolytic streptococci	24	1/4	3/17	7/46	6/71	3/83	3/96				1/100			
Beta-hemolytic streptococci	25	4/16	12/64	9/100										
Enterococci	25									3/12	20/92			2/100

[a] As measured in Mueller-Hinton broth after 24 h of incubation at 37 C.
[b] Inhibitory concentration of cefazolin; expressed as micrograms per milliliter.
[c] Indole-positive.

TABLE 2. *Inhibitory activity of cefazolin against 257 bacterial isolates as determined by the International Collaborative Study agar dilution procedure*[a]

Organism	No. tested	No. of isolates inhibited/cumulative % inhibited												
		0.063[b]	0.125	0.25	0.50	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128	>128
Gram-negative														
Enterobacter sp.	25							1/4			1/8		2/16	21/100
Escherichia coli	25					1/4	4/20	1/24	1/28	4/44	9/80	5/100		
Klebsiella pneumoniae	25					2/8	11/52	4/68	2/76	2/84	1/88	2/96	1/100	
Proteus mirabilis	25							10/40	12/88	3/100				
Proteus sp.[c]	10									1/10		1/20	1/30	7/100
Pseudomonas aeruginosa	25													25/100
Serratia sp.	25												1/4	24/100
Gram-positive														
Staphylococcus aureus	25			4/16	12/64	9/100								
Alpha-hemolytic streptococci	24	5/21	3/33	5/54	5/75	4/92	1/96					1/100		
Beta-hemolytic streptococci	25	1/4	14/60	10/100										
Enterococci	25									1/4	14/60	8/92		2/100

[a] As measured on Mueller-Hinton agar after 24 h of incubation at 37 C.
[b] Inhibitory concentration of cefazolin; expressed as micrograms per milliliter.
[c] Indole-positive.

TABLE 3. Bactericidal activity of cefazolin against 172 isolates of susceptible bacterial isolates[a]

Organism	No. tested[b]	No. of strains killed/cumulative % killed											
		0.063[c]	0.125	0.25	0.50	1.0	2.0	4.0	8.0	16.0	32.0	64.0	128	>128
Gram-negative														
Enterobacter sp.	2							1/50					1/100	
Escherichia coli	23						1/4	1/9	2/17	4/35		4/52	8/87	3/100
Klebsiella pneumoniae	22						3/14	4/32	5/55	3/68	5/91	1/96		1/100
Proteus mirabilis	25							1/4	12/52	6/76	2/84	1/88	1/92	2/100
Proteus sp.[d]	3											1/33		2/100
Gram-positive														
Staphylococcus aureus	25				2/8	6/32	13/84	3/96			1/100			
Alpha-hemolytic streptococci	24	1/4	1/8	6/33	6/58	5/79	4/96							1/100
Beta-hemolytic streptococci	25	4/16	10/56	11/100										
Enterococci	23										3/13	6/39	4/57	10/100

[a] As determined after subculture of negative MIC tubes at 37 C for 24 h.
[b] Number of susceptible strains as determined by the broth dilution method.
[c] Bactericidal concentration of cefazolin; expressed as micrograms per milliliter.
[d] Indole-positive.

tors (2, 5, 8). *Klebsiella* species were the most susceptible of the gram-negative organisms; 64 and 68%, respectively, were inhibited by 4 μg/ml in the broth and agar dilution studies (Tables 1 and 2). Twenty-eight percent of the *E. coli* strains tested were inhibited at 8 μg/ml in both tests. Isolates of *P. mirabilis* also were susceptible, with 72 and 88% being inhibited at 8.0 μg/ml, respectfully, in the broth and agar dilution studies. In contrast, only three of ten isolates of indole-positive *Proteus* species were susceptible to cefazolin at concentrations less than 128 μg/ml; one isolate of *Proteus vulgaris* was inhibited at 16 and 32 μg/ml, respectively, in the agar and broth dilution tests. All isolates of *Pseudomonas aeruginosa*, 24 of 25 isolates of *Serratia*, and 23 of 25 *Enterobacter* species were resistant to 128 μg of cefazolin per ml. Cefazolin was highly active against *S. aurues* and both alpha- and beta-hemolytic streptococci, but poorly active against the enterococci. All 25 isolates of beta-hemolytic streptococci were inhibited by 0.25 μg/ml, and all 25 isolates of *S. aureus* and 23 of 24 isolates of alpha-hemolytic streptococci were inhibited by 2.0 μg/ml in both studies. The enterococci generally were resistant to less than 32 μg/ml. MBCs were determined as part of the broth dilution studies (Table 3). The results of these latter tests indicated that MICs also were bactericidal for the beta-hemolytic streptococci and nearly so for the alpha-hemolytic streptococci. Bactericidal levels were generally twice the MIC value or less for the enterococci, *S. aureus*, *Klebsiella* species, *E. coli*, and *P. mirabilis*.

MICs as determined by the broth and agar dilution procedures generally were in good agreement. Most differences were only twofold or one dilution factor. Statistical analyses (paired *t* test) revealed no significant differences in inhibitory concentrations measured by the two procedures ($P \geq 0.05$) for the following genera: *Klebsiella*, *Proteus*, alpha-hemolytic streptococci, beta-hemolytic streptococci, and enterococci. Differences in MIC values were significant in the case of *S. aureus* ($P < 0.001$) and of borderline significance in the case of *E. coli* ($P < 0.05$). Data for *Enterobacter*, *Pseudomonas*, and *Serratia* were excluded from analyses. This agreement also is demonstrated in Fig. 1, which depicts least-square regression lines for cefazolin MIC values as determined by the agar and broth dilution procedures plotted against paired zones of inhibition obtained with an experimental 30-μg cefazolin disk. This degree of agreement suggests, at least for the cephalosporin antibiotics, that the agar dilution procedure may be used in lieu of broth dilution procedures for testing of large numbers of clinical isolates where the ease, rapidity, and economic advantages of the former method is desired.

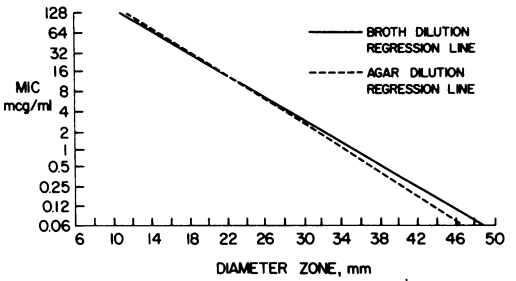

FIG. 1. *Comparison of regression lines for cefazolin MICs as measured by both broth and agar dilution methods with paired zones of inhibition obtained with an experimental 30-μg cefazolin disk.*

As already reported by others, cefazolin appears to equivalent to cephalothin in terms of overall in vitro activity. Cefazolin offers apparent advantages over cephalothin and cephaloridine both in rates of adsorption and in peak serum levels obtainable in man via parenteral administration (5, 7). Cefazolin may also have an advantage over cephaloridine because of its resistance to staphylococcal penicillinase (7). This was observed in a series of experiments using inocula of varying densities. These data, not reported here, showed that with *E. coli* tenfold differences in inoculum size often resulted in fourfold differences or greater in agar dilution end points. Similar changes in inoculum size had a moderate effect on MIC values for penicillin-susceptible and penicillin-resistant strains of *S. aureus;* a tenfold reduction in inoculum size generally was associated with a twofold reduction in the MIC. This effect of inoculum size may explain the differences seen in MIC values obtained with the two dilution methods for *S. aureus* and *E. coli*.

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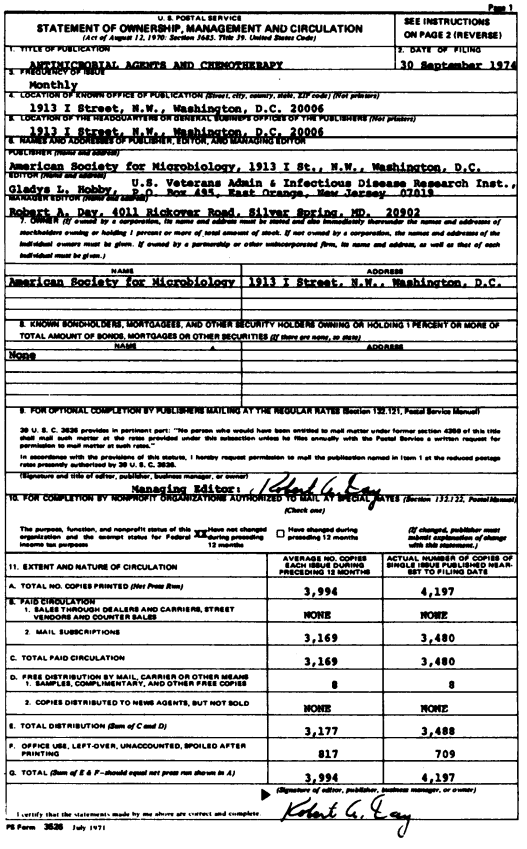

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