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In Vitro Studies with Cefaclor, a New Oral Cephalosporin

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In vitro studies were performed to evaluate the activity of cefaclor in comparison with cephalexin against 180 clinical isolates. Broth dilution susceptibility tests showed cefaclor to be 4- to 16-fold more active than cephalexin against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and cephalothin-susceptible *Enterobacteriaceae*. Neither drug was highly active against cephalothin-resistant *Enterobacteriaceae* or methicillin-resistant *Staphylococcus aureus*. Cefaclor zones with 30-μg disks were generally larger than cephalexin zones, 4 mm larger than cephalothin zones against *Enterobacteriaceae*, and 6 mm smaller than cephalothin zones against *S. aureus*. Quantitative kill curves indicated that killing by both cefaclor and cephalexin was slow and often incomplete over a 24-h period. Cefaclor-induced filamentation of gram-negative bacilli was not as extensive as that produced by cephalexin, and some spherule formation did occur. However, cefaclor was significantly more unstable in solution than cephalexin, with a half-life of less than 6 h at 37°C. Thus, results obtained in tests after prolonged incubation may not provide an accurate measure of cefaclor's activity.

Cefaclor is a new oral cephalosporin with increased activity over cephalexin against many genera of bacteria (2; D. A. Preston, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Ill., Abstr. 352, 1976). The purpose of the current study was to (i) evaluate the antibacterial spectrum and bactericidal activity of cefaclor in comparison with cephalexin against both gram-positive and gram-negative clinical isolates, and (ii) compare results obtained in diffusion tests with 30-μg disks of cephalothin, cephalexin, and cefaclor.

MATERIALS AND METHODS

Antibiotics. Fresh solutions of cefaclor and cephalexin were prepared on the day of use. Cefaclor (compound 99638, Eli Lilly and Co.) was dissolved in 0.1 M KH_2PO_4, pH 4.5, and cephalexin monohydrate (Eli Lilly and Co.) was dissolved in distilled water. Appropriate dilutions of each drug were then made in distilled water or broth media.

Broth dilution tests. Serial twofold broth dilution tests were performed and incubated for 18 to 24 h at 37°C. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of drug inhibiting macroscopic growth. Subcultures to agar plates were made by removing 0.01 ml (calibrated loop) from each clear tube. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of drug preventing all growth on subculture. For staphylococci and *Enterobacteriaceae*, tests were performed in Mueller-Hinton broth (BBL) with a final inoculum of 0.8×10^4 to 1.7×10^4 colony-forming units (CFU)/ml; subcultures were made onto sheep blood agar plates, and incubation was in air. For *Streptococcus pneumoniae*, tests were performed in Todd-Hewitt broth (BBL) with a final inoculum of 0.5×10^4 to 1.7×10^4 CFU/ml; subcultures were made onto blood agar plates, and incubation was in 10% CO_2 in air. For *Haemophilus influenzae*, tests were performed in modified Levinthal broth with a final inoculum of 10^6 CFU/ml; subcultures were made onto chocolate agar, and incubation was in 10% CO_2 in air.

Disk diffusion tests. Disk diffusion tests were performed by the method of Bauer et al. (1). Mueller-Hinton agar plus 5% supplement C (Difco) was used for tests with *H. influenzae*, and Mueller-Hinton agar plus 5% sheep blood was used for tests with *Streptococcus pneumoniae*.

Bactericidal activity. Quantitative kill curves were performed by incubating broth containing antibiotic and bacteria at 37°C for 24 h. Samples were removed at 0, 1, 2, 4, 6, 7, and 24 h, and the number of viable bacteria was determined by dilution plate counts. Tests with *S. pneumoniae* were performed in Todd-Hewitt media; with *H. influenzae*, modified Levinthal media was used; and Mueller-Hinton media was used with all other strains. Results given are averages of duplicate determinations.

Assay for antibiotic activity. The concentration of active antibiotic in solutions of each drug was determined by bioassay. Paper disks impregnated with drug-containing solutions were placed on agar plates that had been inoculated with *Bacillus subtilis* ATCC 6633 according to the Bauer-Kirby procedure (1). After overnight incubation, the sizes of zones of growth inhibition were measured. The concentration of active drug in each test solution was

determined by comparison of zone sizes with results from freshly prepared standards tested simultaneously.

Data analysis. Tests with cephalexin or cephalothin for comparative analysis were performed at the same time as tests with cefaclor. For certain data analysis, *Enterobacteriaceae* were divided into two groups based on results in disk diffusion tests with cephalothin: cephalothin-susceptible – zone size, ≥18 mm; cephalothin-intermediate/resistant – zone size, ≤17 mm.

RESULTS

Broth dilution tests. The in vitro activity of cefaclor and cephalexin was determined against 180 clinical isolates by serial twofold broth dilution tests. The comparative activity of these two drugs based on MICs is shown in Table 1. The activity of cefaclor was equivalent to cephalexin against *Staphylococcus aureus,* and significantly greater than cephalexin against *S. pneumoniae, H. influenzae,* and cephalothin-susceptible *Enterobacteriaceae*. The results for the major genera included in the cephalothin-susceptibile *Enterobacteriaceae* are also shown in Table 1. The comparative activity of the two drugs against these organisms was similar when MBCs were evaluated (Table 2). However, cephalexin was more active than cefaclor against *S. aureus* when MBCs were compared.

The 48 cephalothin-intermediate/resistant *Enterobacteriaceae* included 5 *Klebsiella*, 4 *Enterobacter*, 20 indole-positive *Proteus*, 7 *Escherichia coli*, and 12 *Citrobacter*. Although the percentage of strains inhibited by ≤12.5 μg of cefaclor and cephalexin per ml was 67 and 44, respectively (Table 1), only a few strains were killed by either drug in a concentration of ≤12.5 μg/ml (Table 2). The majority (10 of 14) of the strains killed by ≤12.5 μg of cefaclor per ml and all of the strains killed by ≤12.5 μg of cephalexin per ml were of intermediate susceptibility to cephalothin (Table 3).

In tests with ampicillin-resistant *H. influenzae*, MICs of cefaclor were lower than those of cephalexin; however, MBCs were similar (Table 4). Although MBCs of cefaclor tended to be lower than cephalexin in tests with methicillin-resistant *S. aureus*, MBCs of both drugs were relatively high (Table 5).

The occurrence of discrepancies fourfold or greater between MICs and MBCs of cefaclor varied with the organisms tested. Among the *Enterobacteriaceae*, discrepancies were observed with 52 of 125 strains (42%, Table 6). Most of these discrepancies occurred in tests with cephalothin-intermediate/resistant isolates (29 strains) and *Proteus mirabilis* (19

TABLE 1. *MICs of cefaclor (CCL) and cephalexin (CXN) against 180 clinical isolates*

Organism	No. of strains	Antibiotic	Cumulative % of strains inhibited by (μg/ml):									
			≤0.4	0.8	1.6	3.1	6.2	12.5	25.0	50.0	100.0	>100.0
Staphylococcus aureus	20	CXN	15	65	75	85	90	95	95	95	95	100
		CCL	35	65	80	80	90	95	100			
Streptococcus pneumoniae	15	CXN		13	67	93	100					
		CCL		67	93	100						
Cephalothin-susceptible *Enterobacteriaceae*	77	CXN			13	66	97	100				
		CCL	16	83	99	99	100					
Klebsiella species	25	CXN			36	96	100					
		CCL	88	100								
Escherichia coli	13	CXN				77	100					
		CCL	23	85	92	92	100					
Proteus mirabilis	20	CXN					90	100				
		CCL		70	100							
Salmonella/Shigella	15	CXN			7	93	100					
		CCL	53	100								
Cephalothin-intermediate/resistant *Enterobacteriaceae*	48	CXN			2	8	17	44	63	77	81	100
		CCL		4	19	33	56	67	75	79	83	100
Haemophilus influenzae	20	CXN	5	15	35	90	100					
		CCL	60	90	100							

TABLE 2. *MBCs of cefaclor (CCL) and cephalexin (CXN) against 180 clinical isolates*

Organism	No. of strains	Antibiotic	Cumulative % of strains killed by (μg/ml):										
			≤0.4	0.8	1.6	3.1	6.2	12.5	25.0	>25.0[a]	50.0[b]	100.0[b]	>100.0[b]
Staphylococcus aureus	20	CXN	5	30	60	75	80	80	80		80	85	100
		CCL			5	30	40	65	80		90	100	
Streptococcus pneumoniae	15	CXN				13	67	100					
		CCL		27	93	93	100						
Cephalothin-susceptible *Enterobacteriaceae*	77	CXN			5	61	78	99	100				
		CCL		27	62	84	100						
Klebsiella species	25	CXN			16	96	100						
		CCL	28	88	100								
Escherichia coli	13	CXN				69	100						
		CCL		38	92	92	100						
Proteus mirabilis	20	CXN					15	95	100				
		CCL				45	100						
Salmonella/Shigella	15	CXN				80	100						
		CCL		53	73	100							
Cephalothin-intermediate/resistant *Enterobacteriaceae*	48	CXN			2	4	15	19	31		40	56	100
		CCL			2	15	19	29	42		52	58	100
Haemophilus influenzae	20	CXN			5	30	60	85	95	100			
		CCL	10	40	55	75	90	90	95	100			

[a] Applies only to *H. influenzae*.
[b] Applies to all strains except *H. influenzae*.

TABLE 3. *Bactericidal activity of cefaclor (CCL) and cephalexin (CXN) against cephalothin-intermediate and -resistant Enterobacteriaceae*

Enterobacteriaceae	No. of strains tested	% of strains killed by ≤12.5 μg/ml	
		CCL	CXN
Cephalothin intermediate[a]	17	59	53
Cephalothin resistant[b]	31	13	0

[a] Zone size, 15 to 17 mm.
[b] Zone size, ≤14 mm.

TABLE 4. *Activity[a] of cefaclor and cephalexin against ampicillin-resistant Haemophilus influenzae[b]*

H. influenzae strain	Cefaclor		Cephalexin	
	MIC	MBC	MIC	MBC
31	0.8	3.1	3.1	3.1
14	0.4	>25.0	3.1	25.0
15	0.2	3.1	1.6	6.2
16	0.2	6.2	1.6	3.1
27	1.6	25.0	6.2	>25.0

[a] All results in micrograms per milliliter.
[b] Tests performed in modified Levinthal broth.

TABLE 5. *Activity[a] of cefaclor and cephalexin against methicillin-resistant Staphylococcus aureus*

S. aureus strain	Cefaclor		Cephalexin	
	MIC	MBC	MIC	MBC
22	12.5	50	6.2	>100
23	6.2	50	3.1	100
25	6.2	100	12.5	>100
27	25	25	>100	>100

[a] All results are in micrograms per milliliter.

strains). Discrepancies occurred in tests with 19 of 20 staphylococci (including the four methicillin-resistant strains) and 8 of 20 *H. influenzae* (including the five ampicillin-resistant strains). No discrepancies occurred in tests with *S. pneumoniae*. Although fewer discrepancies were observed in tests with cephalexin (Table 6), MBCs of this drug were still higher in general than MBCs of cefaclor (Table 2).

Disk diffusion tests. Disk diffusion tests were performed on 179 clinical isolates with 30-μg disks of cefaclor, cephalexin, and cephalothin (Table 7). Cefaclor zones tended to be (i) larger than cephalothin zones in tests with *Enterobacteriaceae,* (ii) smaller than cephalothin zones in tests with *S. aureus* and *S. pneumoniae,* (iii) equivalent to cephalothin zones in tests with *H. influenzae,* and (iv) larger than cephalexin zones in tests with most isolates. The smallest cefaclor zones were observed with strains of cephalothin-intermediate/resistant *Enterobacteriaeceae* (13 mm) and methicillin-resistant staphylococci (8 mm).

Results obtained in disk diffusion tests were compared with those obtained in broth dilution tests with cefaclor. As cefaclor MICs increased, sizes of zones of growth inhibition tended to decrease (Fig. 1). A second analysis based on MBCs revealed a similar trend; i.e., as MBCs increased, zone sizes tended to decrease (Fig. 2). Applying the same criteria for interpreting disk results with cefaclor as are used with cephalothin, a comparison was made to determine which disk most accurately reflected an organism's susceptibility to cefaclor (Table 8). Among those organisms determined to be susceptible to cefaclor (MIC ≤12.5 μg/ml), more strains gave zone sizes ≥18 mm with cefaclor disks than with cephalothin disks. The difference, however, was not significant (chi-square, Yates correction, $P > 0.1$). Among organisms determined to be resistant to cefaclor (MIC > 12.5 μg/ml), similar numbers gave zone sizes of ≤14 mm with either disk. Disagreement between the results of the disk diffusion and broth dilution tests (i.e., susceptible in one, resistant in the other) was similar regardless of which disk was used.

Bactericidal activity. Quantitative kill curves were performed with cefaclor and cephalexin to determine the rate and completeness of killing by each drug. Results of tests performed with each drug in a concentration of 10 μg/ml are shown in Fig. 3. Except in tests with *S. pneumoniae* (Fig. 3E), killing did not begin with either drug until growth began in the drug-free control tubes, and was usually incomplete at 24 h. The rate of killing of *H. influ-*

TABLE 6. *Occurrence of discrepancies fourfold or greater between MICs and MBCs of cefaclor (CCL) and cephalexin (CXN)*

Organism	No. of strains tested	No. (%) of strains with discrepancies[a]	
		CCL	CXN
All *Enterobacteriaceae*	125	52 (42)	21 (17)
Cephalothin susceptible	77	23 (30)	0
Cephalothin intermediate/resistant	48	29 (61)	21 (44)
S. aureus	20	19 (95)	6 (30)
H. influenzae	20	8 (40)	9 (45)
S. pneumoniae	15	0	2 (13)
All strains	180	79 (44)	38 (21)

[a] MBC/MIC ≥ 4.

TABLE 7. *Results of disk diffusion tests with 30-μg disks of cefaclor (CCL), cephalexin (CXN), and cephalothin (CF) against gram-positive and -negative clinical isolates*

Organism	No. of strains	CCL zone size (mm)		CXN zone size (mm)		CF zone size (mm)	
		Avg	Range	Avg	Range	Avg	Range
CF-susceptible *Enterobacteriaceae*	77	26	19–32	20	16–26	22	18–30
CF-intermediate/resistant *Enterobacteriaceae*	48	13	6–23	11	6–21	11	6–17
S. aureus							
Methicillin susceptible	16	23	15–32	25	18–29	28	24–35
Methicillin resistant	4	8	8–9	6	6	22	14–26
S. pneumoniae	14	31	24–36	27	22–33	34	25–41
H. influenzae							
Ampicillin susceptible	15	23	20–28	17	13–23	23	18–26
Ampicillin resistant	5	21	18–22	15	14–16	18	16–20

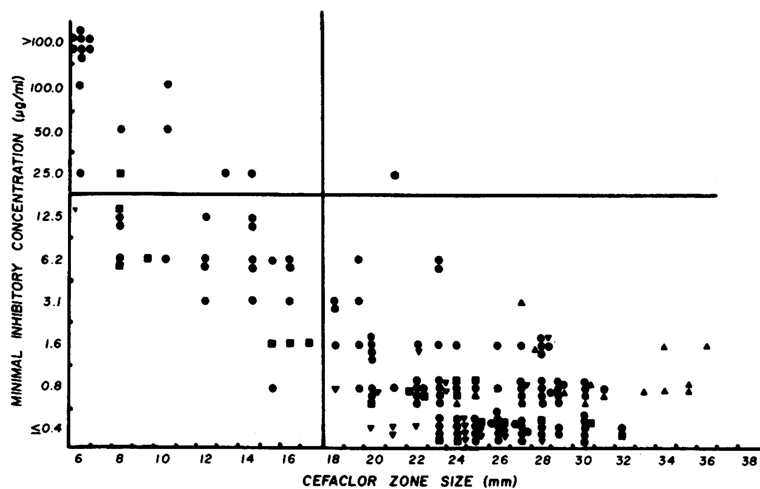

FIG. 1. *Comparison of MICs of cefaclor and zone sizes obtained with 30-μg disks. Enterobacteriaceae (●), Staphylococcus aureus (■), Haemophilus influenzae (▼), Streptococcus pneumoniae (▲).*

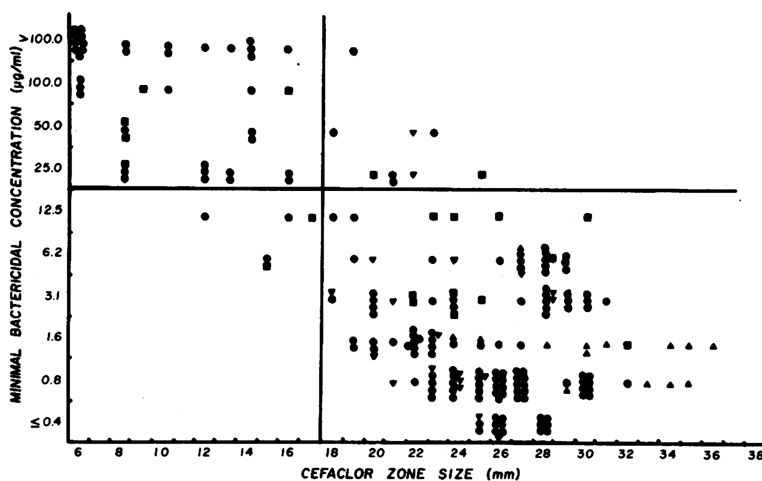

FIG. 2. *Comparison of MBCs of cefaclor and zone sizes obtained with 30-μg disks. Enterobacteriaceae (●), Staphylococcus aureus (■), Haemophilus influenzae (▼), Streptococcus pneumoniae (▲).*

enzae by cefaclor and cephalexin was identical (Fig. 3A). Because of this slow and incomplete killing of relatively susceptible organisms (MBC, 0.4 to 3.1 μg/ml), several tests were repeated using 30 μg of each drug per ml (Fig. 4). As with the lower concentration, killing usually proceeded slowly between 2 and 24 h; however, complete killing was achieved in all tests with cefaclor by 24 h. Gram stains were performed on sediments from centrifuged samples removed from tests containing 10 μg of each drug per ml after 4 h of incubation. Cephalexin-treated staphylococci were two to four times larger than control cells, and many daughter cells were incompletely separated. Cefaclor-treated staphylococci were similar in appearance to control cells. Cephalexin-treated gram-negative bacilli showed extensive filamentation; the length of some filaments was equivalent to the length of 100 to 150 control cells. Some filamentation was observed among cefaclor-treated gram-negative bacilli, but the average length of cefaclor-induced filaments was much shorter (20 to 50 cells), and some spherule formation was also observed. Cephalexin-induced filaments stained darkly gram negative, as did the control cells, whereas many cefaclor-induced filaments were mottled or appeared to be ghost cells.

An additional explanation for the slow bactericidal effect and the frequent discrepancies between MICs and MBCs of cefaclor was instability of the drug in solution. Were the drug to degrade significantly during the tests, results

TABLE 8. *Susceptibility of clinical isolates to cefaclor as determined in broth dilution tests and compared with results obtained in diffusion tests with 30-μg cefaclor (CCL) or cephalothin (CF) disks*

Cefaclor broth dilution result	No. of isolates	Disk diffusion result[a]					
		No. (%) susceptible		No. intermediate		No. (%) resistant	
		CCL	CF	CCL	CF	CCL	CF
Susceptible by MIC[b]	162	138 (85)	127 (79)	8	16	16 (10)	19 (12)
Resistant by MIC[c]	17	1 (6)	0 (0)	0	1	16 (94)	16 (94)

[a] Susceptible, ≥18 mm; intermediate, 17 to 15 mm; resistant, ≤14 mm.
[b] MIC of ≤12.5 μg/ml.
[c] MIC of >12.5 μg/ml.

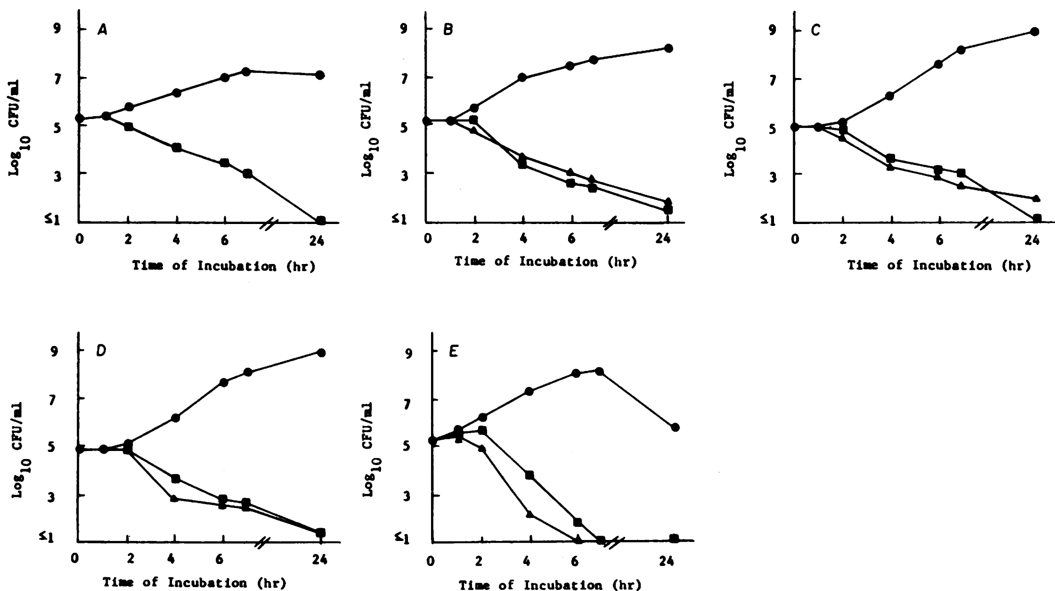

FIG. 3. *Rate of bacterial killing by 10 μg of cefaclor (▲) and cephalexin per ml (■). (A) Haemophilus influenzae (results for the two drugs were identical); (B) Staphylococcus aureus; (C) Proteus mirabilis; (D) Klebsiella species; (E) Streptococcus pneumoniae; drug-free control (●).*

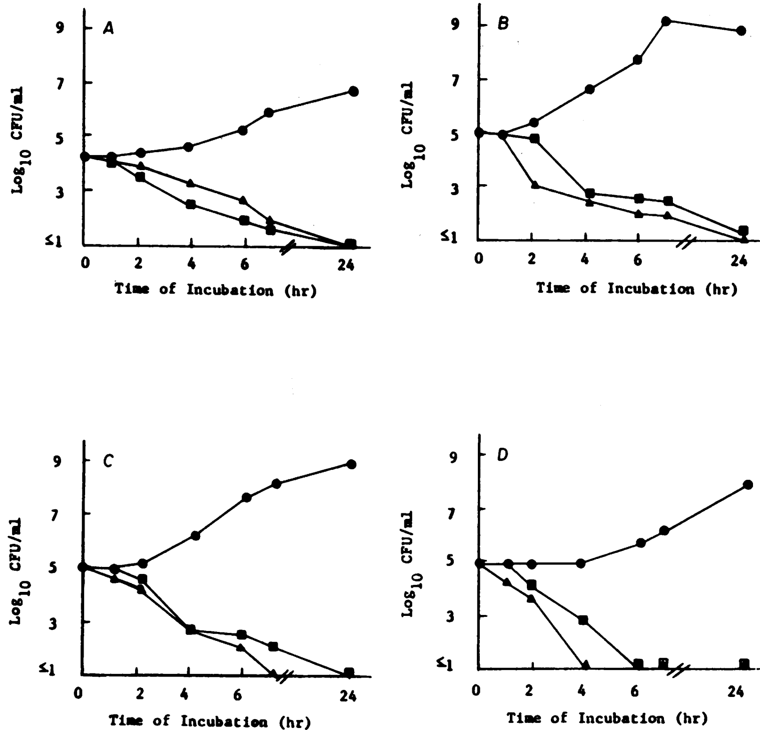

FIG. 4. *Rate of bacterial killing by 30 μg of cefaclor (▲) and cephalexin per ml (■). (A) Staphylococcus aureus; (B) Klebsiella sp.; (C) Proteus mirabilis; (D) Streptococcus pneumoniae; drug-free control (●).*

TABLE 9. *Stability of cefaclor and cephalexin in Mueller-Hinton broth incubated at 37°C*

Time of incubation (h)	Cefaclor		Cephalexin	
	Active concn (μg/ml)	% Loss	Active concn (μg/ml)	% Loss
0	10		10	
1	10	0	10	0
2	10	0	7	30
4	5.6	44	7	30
6	4.0	60	7	30
7	2.8	72	7	30
24	0.1	99	6	40

obtained at 24 h might be artifactually high due to regrowth of surviving bacteria. Therefore, the stability of cefaclor and cephalexin in Mueller-Hinton broth was determined by incubating each drug for 24 h at 37°C in air. At various time intervals, portions were removed and the concentration of active drug was determined by bioassay. As shown in Table 9, cefaclor degraded significantly by 4 h; less than half remained as active drug after 6 h, and virtually all had degraded by 24 h. Although cephalexin also degraded, the rate and extent was not as striking.

DISCUSSION

Results of this study suggest that cefaclor is significantly more active than cephalexin against *S. pneumoniae*, *H. influenzae*, and cephalothin-susceptible *Enterobacteriaceae*. Results of disk diffusion tests suggest that 30-μg cephalothin disks may be used to determine the susceptibility of a strain to cefaclor, even though cephalothin zones tended to be smaller than cefaclor zones. The percentage of strains resistant to cefaclor in both broth dilution tests and disk diffusion tests was similar regardless of which disk was used. The major difference between results with the two disks was observed in tests with strains susceptible to cefaclor in broth dilution assays. A higher percentage of these strains gave zone sizes of ≥18 mm with cefaclor disks than with cephalothin disks; however, this difference was not significant.

Bacterial killing by cefaclor was found to be slow and usually incomplete by 24 h. However, a similar slow rate was observed with cephalexin. The slow bactericidal effect of cephalexin that has been reported previously by Muggleton et al. (5) may be due to the extensive filamentation produced by this drug over a broad concentration range (3–6). Although some fila-

mentation of gram-negative bacilli did occur with cefaclor, it was not as extensive as that produced by cephalexin, and some spherules were observed. Furthermore, most cefaclor-treated staphylococci were normal in appearance. Thus, the great instability of cefaclor in solution may be a major factor contributing to the slow bactericidal effect. As noted in tests with cephalothin (7), results obtained after prolonged incubation may not provide an accurate measure of a drug's activity if it is relatively unstable in solution.

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