ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1975, p. 128-132

Vol. 7, No. 2
Printed in U.S.A.

Susceptibility of Anaerobes to Cefoxitin and Other Cephalosporins

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Received for publication 12 August 1974

The in vitro susceptibility of 155 strains of anaerobic bacteria to five cephalosporin antibiotics was tested. Cefoxitin was the most active against 33 isolates of *Bacteroides fragilis*; 82% of the strains were sensitive at 16 μg/ml. At 64 μg/ml cefazolin and cephaloridine were also generally effective. Cephalothin and cephalexin were relatively inactive versus *B. fragilis*. Cefoxitin, cephaloridine, cefazolin, and cephalothin showed comparable activity against 122 strains of anaerobes other than *B. fragilis*. More than 90% of the strains were sensitive to each of these antimicrobials at 16 μg/ml. Cephalexin was the least effective cephalosporin against all species tested.

In a recent review of anaerobic bacteria in serious infections in man, it was noted that *Bacteroides fragilis* occupied a preeminent position because of its frequency of isolation and its resistance to commonly used antimicrobial agents (6). At present, the armamentarium for treating infections with this organism is limited to clindamycin and chloramphenicol. Serious complications with both of these antimicrobials has led to a search for alternate agents.

Several laboratories have assessed the activity of cephalosporins against *B. fragilis*. Martin et al. studied 195 strains of *B. fragilis* and found that only 4% were susceptible to cephalothin at 12.5 μg/ml, and 9% were susceptible at the 25 μg/ml level (11). Kislak, in a study of 40 strains of *B. fragilis*, showed that cephaloridine had increased activity; however, 50% of the strains were still resistant at 25 μg/ml (9). The clinical significance of these in vitro observations was demonstrated in a double-blind study of antimicrobial treatment in intra-abdominal sepsis following trauma (14). There was a significant increase in the isolation of anaerobes in patients treated with cephalothin compared to those receiving clindamycin. The increased incidence of septic complications in the cephalothin treated patients was attributed to anaerobic infections, in which *B. fragilis* was the most common isolate.

Previous reports have suggested that the resistance of *B. fragilis* to cephalosporin antibiotics is due to the production of cephalosporinase (1, 4). The development of cephamycin C, which is known to be resistant to certain beta-lactamases, raised speculation that this agent might have superior activity against *B. fragilis* (3, 12). In a limited report, Kosmidis et al. (10) showed that cefoxitin had greater activity than cephalothin against 10 strains of *B. fragilis*. The purpose of this study was to examine the in vitro antimicrobial activity of cefoxitin against a miscellany of anaerobic bacteria including *B. fragilis* and to compare the results with four other cephalosporin antibiotics.

MATERIALS AND METHODS

Bacterial strains. The sample included 155 strains of various species of anaerobic bacteria cultured in the Anaerobic Research Laboratory, Sepulveda Veterans Administration Hospital, from clinical infections taken between May 1973 and March 1974. The strains were isolated by anaerobic chamber techniques and identified according to the criteria of Holdeman and Moore (8).

Antibiotics. Cephalothin, cephaloridine, and cephalexin were supplied by Eli Lilly & Co. (Indianapolis, Ind.); cefazolin was supplied by Smith Kline & French Laboratories (Philadelphia, Pa.); and cefoxitin was supplied by Merck Institute (Rahway, New Jersey).

Procedures. The procedure used for the antimicrobial susceptibility testing was a modified agar dilution method utilizing a Steers replicator (13). Eight to ten fresh colonies of each isolate were inoculated into prereduced brain heart infusion broth supplemented with yeast extract (0.5%), hemin (0.0005%), and vitamin K_1 (0.1 μg/ml; Scott Laboratories, Fiskeville, R.I.). The inoculum was incubated at 37 C; rapid growers were cultured for 5 to 6 h, and slow growers were cultured for 24 h. The agar medium consisted of brain heart infusion base (BBL) with 1.5% agar enriched with 5% laked sheep blood and vitamin K_1 (10 μg/ml). The plates were prepared on

the morning of the test by adding the blood and serial twofold dilutions of the antibiotic to a final volume of 20 ml; the final mixture was poured into petri dishes (100 by 15 mm). The final concentrations of the antibiotics ranged from 0.125 to 64 μg/ml. After drying in an incubator for 45 min, the plates were transferred into the anaerobic chamber 3 to 4 h prior to inoculating.

The inoculum was diluted (1:100) to give a concentration of approximately 10^7 colony-forming units per ml, and the plates were inoculated with approximately 10^4 colony-forming units per ml in the chamber with a Steers replicator. Anaerobic and aerobic control plates were included for each antimicrobial agent. The plates were incubated for 48 h at 37 C in the anaerobic chamber. The minimal inhibitory concentration was defined as the lowest concentration of antibiotic which showed no growth, a barely visible haze, or a single colony (5). A control strain of *B. fragilis* (Wadsworth Anaerobic Laboratory no. 1887; kindly supplied by V. L. Sutter) was included in each test run.

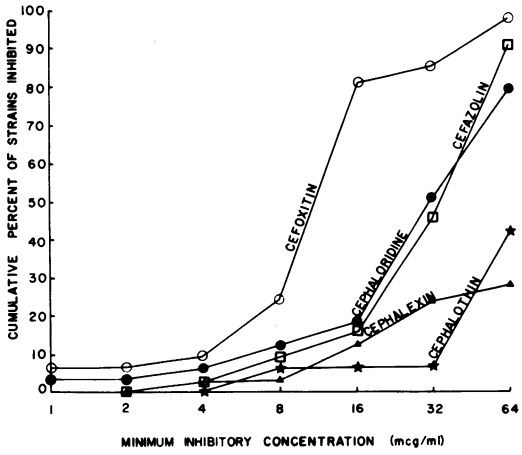

FIG. 1. *The susceptibility of Bacteroides fragilis to cefoxitin, cefazolin, cephaloridine, cephalothin, and cephalexin.*

RESULTS

The in vitro activity of the five antimicrobial agents against 33 strains of *B. fragilis* demonstrates that cefoxitin is the most active compound (Fig. 1). At 16 μg/ml, 82% of the strains were inhibited, and at 64 μg/ml all but one were inhibited. Cefazolin and cephaloridine possessed similar activity against *B. fragilis*, although these drugs were generally fourfold less active than cefoxitin. Cephalexin and cephalothin showed the poorest activity; only 27 and 42% of strains were susceptible at 64 μg/ml, respectively.

Cefoxitin also demonstrated good activity against anaerobes other than *B. fragilis*. Except for two strains of fusobacteria all other gram-negative anaerobes were inhibited by 16 μg/ml (Table 1). Among 43 strains of gram-negative cocci 42 were inhibited by 16 μg/ml. All ten strains of *Eubacterium* were inhibited by 16 μg/ml or less. *Clostridium perfringens* strains were inhibited by 4 μg/ml or less, whereas 81% of the other *Clostridium* species were inhibited by 16 μg/ml. Cefoxitin was effective against all clostridia at the 64 μg/ml level.

With cefazolin, all but one of the gram-negative bacilli other than *B. fragilis* were inhibited by 16 μg/ml (Table 2). All the anaerobic cocci except three strains of *Peptostreptococcus intermedius* were inhibited by 16 μg/ml or less. The 19 gram-positive non-spore-forming rods were inhibited by 64 μg/ml, and the 31 clostridia were inhibited by 32 μg of cefazolin per ml.

At 16 μg/ml, cephaloridine shows generally excellent activity against all the strains of anaerobic bacteria tested except *B. fragilis* (Table 3). Occasional resistant strains included one *Fusobacterium varium*, three *P. intermedius*, and one *Clostridium ramosum*.

Cephalothin at 16 μg/ml had moderately good activity against most strains other than *B. fragilis* (Table 4). At 64 μg/ml, two strains of *F. varium* and three strains of *Clostridium* species were resistant.

Cephalexin showed the poorest activity against the 155 strains (Table 5). At 16 μg/ml, 37% of the anaerobic cocci, 45% of fusobacteria, and 61% of clostridia were resistant.

The control strain *B. fragilis* (Wadsworth Anaerobic Laboratory no. 1887) was within the previously performed limits of susceptibility in all the tests (V. L. Sutter, personal communication).

DISCUSSION

The results of this study indicate that of the five cephalosporin antimicrobial agents tested, cefoxitin was the most consistently active against the 155 anaerobic strains. With conventional doses, 16 μg/ml was considered the upper limit of the susceptible range, based on readily achievable blood levels (2). Cefoxitin showed greater activity at this level against the 33 strains of *B. fragilis* as compared to the other agents. Higher doses of cefoxitin, cephalothin, and cefazolin can produce blood levels of 64 μg/ml (2, 7, 10). All but one strain of *B. fragilis* was inhibited by cefoxitin at this level, compared to 90% of strains that were inhibited by cefazolin and less than 50% by cephalothin.

TABLE 1. *Comparison of susceptibility of anaerobic bacteria to cefoxitin*

Organism	No. strains tested	Cumulative % susceptible to indicated concn (μg/ml)								
		0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
Bacteroides fragilis	33		3	6		9	24	82	85	97
Bacteroides sp.	9		33	89		100				
Fusobacterium sp.	11		46	55		64	73	82		
Peptococcus	19	21	53	74	89	100				
Peptostreptococcus	24		17	33	54	67	83	96		
Veillonella	9	11	50	74	100					
Eubacterium	10			10	20	40	50	100		
Nonsporing gram-positive rods	3		33						67	
Propionibacterium acnes	6	17	83	100						
Clostridium perfringens	15	7	53	67	93	100				
Clostridium sp.	16		25	44	50	69	75	81	87	100

TABLE 2. *Comparison of susceptibility of anaerobic bacteria to cefazolin*

Organism	No. strains tested	Cumulative % susceptible to indicated concn (μg/ml)								
		0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
Bacteroides fragilis	33					3	9	15	45	91
Bacteroides sp.	9	22	67	89	100					
Fusobacterium sp.	11	27	55	64				91		
Peptococcus	19	37	47	58	84	95	100			
Peptostreptococcus	24	13	42	50	71	79		83	96	100
Veillonella	9	22	78	100						
Eubacterium	10	10		20		40		50	70	100
Nonsporing gram-positive rods	3		33					67		
Propionibacterium acnes	6		50	100						
Clostridium perfringens	15	7	20	47	80	93	100			
Clostridium sp.	16	13	31	44	50	56	75	86	100	

TABLE 3. *Comparison of susceptibility of anaerobic bacteria to cephaloridine*

Organism	No. strains tested	Cumulative % susceptible to indicated concn (μg/ml)								
		0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
Bacteroides fragilis	33			3		6	12	18	52	79
Bacteroides sp.	9	78	89	100						
Fusobacterium sp.	11	36	64					82		
Peptococcus	19	21	47	79	90	95	100			
Peptostreptococcus	24	25	46	67	75	86			100	
Veillonella	9	78	100							
Eubacterium	10	10	20	40	50	70	100			
Nonsporing gram-positive rods	3		33		67			100		
Propionibacterium acnes	6	83	100							
Clostridium perfringens	15		33	80	100					
Clostridium sp.	16	19	44	69	75	81		94	100	

Cefoxitin also proved generally effective against other anaerobic bacteria. Among 122 strains of various anaerobes other than *B. fragilis*, over 90% were susceptible at 16 μg/ml. With these strains the activities of cefoxitin, cephaloridine, cefazolin, and cephalothin were quite comparable. Cephalexin, however, was consistently less active than the other cephalosporins. A significant portion of nearly all anaerobic species tested proved resistant to cephalexin at easily achievable blood levels.

It is of interest that cefoxitin demonstrated

TABLE 4. *Comparison of susceptibility of anaerobic bacteria to cephalothin*

Organism	No. strains tested	Cumulative % susceptible to indicated concn (μg/ml)								
		0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
Bacteroides fragilis	33						6			42
Bacteroides sp.	9	44	89		100					
Fusobacterium sp.	11	36	55			64			73	82
Peptococcus	19	26	42	63	79	95			100	
Peptostreptococcus	24	29	46	67	79	83		86	92	100
Veillonella	9	67	78		89	100				
Eubacterium	10	10	20	40			70	80	90	100
Nonsporing gram-positive rods	3	33							100	
Propionibacterium acnes	6	50	100							
Clostridium perfringens	15		7		20	60	87	100		
Clostridium sp.	16		6	19	38	50	63	75	81	

TABLE 5. *Comparison of susceptibility of anaerobic bacteria to cephalexin*

Organism	No. strains tested	Cumulative % susceptible to indicated concn (μg/ml)								
		0.1	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
Bacteroides fragilis	33					3		12	24	27
Bacteroides sp.	9	11	67		89				100	
Fusobacterium sp.	11		9	36	55				64	
Peptococcus	19		10	26	37	47	53	63	89	95
Peptostreptococcus	24	8	13	17	25	46	50	63	71	75
Veillonella	9		44	67	90	100				
Eubacterium	10				30	40	50	90	100	
Nonsporing gram-positive rods	3							33	67	
Propionibacterium acnes	6		17	83	100					
Clostridium perfringens	15						7	40	93	100
Clostridium sp.	16					6	13	38	56	63

activity against the gram-positive anaerobes which was comparable to that of the other cephalosporins. This is in contrast to the activity of this agent against facultative gram-positive organisms, such as the staphylococcus and pneumococcus (15).

The excellent activity of cefoxitin against several species of anaerobic bacteria, including *B. fragilis*, would suggest that it may be useful in the treatment of anaerobic infections. Cefazolin, at higher dosages, may also be efficacious on the basis of in vitro studies. Alternative drugs for anaerobes would be desirable, and it may be warranted to test these cephalosporin antibiotics in anaerobic infections.

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