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Comparative In Vitro Activity of Newer Cephalosporins Against Anaerobic Bacteria

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The in vitro susceptibilities of 408 recent clinical isolates of anaerobic bacteria against cefaclor, cephalexin, cephalothin, cefazolin, cefamandole, and cefoxitin were compared by an agar dilution technique. Against gram-positive bacteria, especially peptococci, peptostreptococci, and propionibacteria, cephalexin and cefaclor were significantly less active than cephalothin ($P < 0.05$). Cephalexin was also less active than cephalothin against clostridia and lactobacillus ($P < 0.05$). Against gram-negative bacteria, major differences were observed primarily with *Bacteroides fragilis*, against which cephalexin, cefazolin and cefoxitin were all significantly more active than cephalothin ($P < 0.001$). At concentrations of 16 μg per ml, however, all cephalosporins showed high in vitro activity, except against *Lactobacillus* species and *B. fragilis*. Cephalothin, cefazolin and cefamandole were considerably more active against the former, whereas cefoxitin was distinctly more active against the latter.

A host of new cephalosporin and cephamycin antibiotics have been introduced recently, some differing considerably both in antibacterial spectra and pharmacological properties. Their comparative in vitro activity against anaerobic bacteria, however, has not been extensively studied. In this study, we examined the in vitro susceptibility of 408 recent clinical isolates of anaerobic bacteria by an agar dilution technique against six cephalosporins, including cefaclor, cephalexin, cephalothin, cefazolin, cefamandole, and cefoxitin.

MATERIALS AND METHODS

The isolates examined in this study, obtained from inpatients of Harbor General Hospital during 1974 through 1977, are listed in Table 1. Identification to species level was determined in prereduced anaerobically sterilized differential media by the method of Holdeman and Moore (6). Clinical isolates were stored in 20% skim milk and frozen at −75°C until ready for susceptibility testing.

Susceptibility testing was performed by an agar-dilution technique previously described by us (3). A reference strain with known minimal inhibitory concentrations (MICs) was included in each test to demonstrate reproducibility, and the MIC recorded was the least antibiotic concentration that yielded no visible growth after 48 h of incubation.

RESULTS

The geometric mean MIC and cumulative percent of various anaerobic bacteria inhibited by each cephalosporin antibiotic are summarized in Tables 2 and 3.

Against gram-positive anaerobic bacteria, cephalexin and cefaclor were significantly less active than cephalothin ($P < 0.05$), especially against *Peptococcus, Peptostreptococcus,* and *Propionibacterium* species. Cephalexin was uniquely less active than cephalothin against *Clostridium* ($P < 0.001$) and *Lactobacillus* species ($P < 0.05$). At concentrations of 16 μg per ml, however, all cephalosporins inhibited gram-positive anaerobic bacteria except the *Lactobacillus* species relatively well.

Against gram-negative anaerobic bacteria, major differences in in vitro activity of the cephalosporins were observed primarily with *Bacteroides fragilis*. Cephalexin, cefazolin, and cefoxitin were all significantly more active than cephalothin ($P < 0.001$). Cefoxitin was the most active, followed by cefazolin and cephalexin. Cefamandole, cefaclor and cephalothin were least active. All cephalosporins showed good in vitro activity against gram-negative anaerobes other than *B. fragilis*, although their geometric mean MICs against *Bacteroides* were generally two-

TABLE 1. *Genera and species of 408 anaerobic bacteria tested*

Organism	No. tested	Organism	No. tested
Peptococcus asaccharolyticus	21	*Veillonella parvula*	18
P. prevotii	13	*V. alcalescens*	5
P. saccharolyticus	6	*Acidaminococcus fermentans*	2
P. constellatus	5	*Bacteroides fragilis* subsp. *vulgatus*	16
P. magnus	3	*B. fragilis* subsp. *distasonis*	13
P. variabilis	2	*B. fragilis* subsp. *fragilis*	10
Peptostreptococcus micros	28	*B. fragilis* subsp. *thetaiotaomicron*	6
P. anaerobius	14	*B. fragilis* subsp. *ovatus*	3
P. intermedius	6	*B. fragilis* subsp. "other"	3
Clostridium perfringens	17	*Bacteroides oralis*	11
C. cadaveris	2	*B. pneumosintes*	8
C. innocuum	2	*B. amylophilus*	6
C. ramosum	2	*B. ruminicola*	6
C. aminovalericum	1	*B. capillosus*	4
C. botulinum	1	*B. clostridiiformis*	4
C. glycolicum	1	*B. corrodens*	4
C. scatologenes	1	*B. melaninogenicus*	3
Actinomyces naeslundii	8	*B. nodosus*	3
A. viscosus	1	*B. coagulans*	2
Eubacterium lentum	6	*B. biacutus*	1
E. aerofaciens	3	*B. furcosus*	1
E. alactolyticum	1	*Fusobacterium nucleatum*	8
E. tortuosum	1	*F. naviforme*	3
Lactobacillus acidophilus	5	*F. mortiferum*	2
L. fermentum	4	*F. russii*	2
L. catenaforme	1	*F. gonidiaformans*	1
L. leichmannii	1	*F. necrophorum*	1
Bifidobacterium adolescentis	4	*F. prausnitzii*	1
B. breve	4	*F. varium*	1
B. bifidum	2	*Campylobacter fetus*	6
B. infantis	1	*C. sputorum*	2
Propionibacterium acnes	81		
P. granulosum	4		

to fivefold higher than against *Fusobacterium* or *Veillonella* species.

DISCUSSION

Data presented here show that in the study reported herein, except for *B. fragilis* and *Lactobacillus* species, only minor differences existed in the in vitro activity of the cephalosporins tested against anaerobic bacteria. Most were readily inhibited by 16 μg of each antibiotic per ml.

Against *Lactobacillus* species, the geometric mean MICs of cefaclor and cephalexin were fivefold higher than that of cephalothin, and only 45% of strains were inhibited by 16 μg of either of these two antibiotics per ml. Cefoxitin was similarly inactive. Although cephalothin exhibited good inhibitory activity in vitro against *Lactobacillus* species, we have found that the bactericidal activity of both cephalothin and penicillin G are often widely disparate, with the minimal bactericidal concentration ≥100-fold more than the corresponding MIC (2). This suboptimal bactericidal activity in vitro is reflected in the frequency of therapeutic failures of these antibiotics in eradicating lactobacillemia associated with endocarditis (2).

The most striking differences in in vitro activity between the cephalosporins were observed with *B. fragilis*. Cefoxitin was by far the most active; 70% of strains were inhibited by 16 μg of the antibiotic per ml. This is similar to the results reported by Sutter and Finegold (7) and Ernst et al. (5). Cefoxitin is a cephamycin derivative known to be resistant to certain beta-lactamases as well as cephalosporinase of *B. fragilis* (1, 4, 8). In contrast, cefamandole, another cephalosporin resistant to hydrolysis by beta-lactamases of facultative bacteria (5), is relatively inactive against *B. fragilis*, as are cefaclor and cephalothin.

TABLE 2. *Comparative in vitro activity of cephalosporins against gram-positive anaerobic bacteria*

Organism tested against various agents (no. of isolates)	Geometric mean MIC ± SD (μg/ml)[a]	Cumulative % strains inhibited by (μg/ml):				
		≤0.25	1	4	16	64
Peptococcus (50)						
Cephalothin	0.45 ± 0.29	52	94	96	100	
Cephalexin	0.80 ± 0.55[b]	50	68	86	96	100
Cefaclor	0.94 ± 0.50[b]	32	68	86	96	100
Cefazolin	0.37 ± 0.26	64	96	98	100	
Cefamandole	0.54 ± 0.28	42	94	98	100	
Cefoxitin	0.58 ± 0.42	56	78	92	100	
Peptostreptococcus (48)						
Cephalothin	0.48 ± 0.36	60	87	94	100	
Cephalexin	0.84 ± 0.50[c]	42	69	85	100	
Cefaclor	1.36 ± 0.59[d]	33	52	79	92	100
Cefazolin	0.45 ± 0.34	60	89	96	100	
Cefamandole	0.42 ± 0.34	69	85	96	100	
Cefoxitin	0.68 ± 0.46	50	73	85	100	
Clostridium (27)						
Cephalothin	1.16 ± 0.42	18	63	85	96	100
Cephalexin	5.16 ± 0.67[d]	4	26	55	81	92
Cefaclor	2.0 ± 0.70	22	48	74	81	100
Cefazolin	0.94 ± 0.51	33	74	81	100	
Cefamandole	0.90 ± 0.40	30	63	92	96	100
Cefoxitin	1.58 ± 0.54	7	67	81	85	100
Actinomyces (9)						
Cephalothin	2.15 ± 1.21	44	44	67	78	89
Cephalexin	1.35 ± 0.98	55	55	67	89	100
Cefaclor	2.0 ± 0.83	33	44	55	89	100
Cefazolin	1.70 ± 1.10	44	55	67	89	89
Cefamandole	1.16 ± 0.80	55	55	78	89	100
Cefoxitin	1.84 ± 0.92	33	55	55	78	100
Eubacterium (11)						
Cephalothin	0.72 ± 0.68	64	73	91	91	100
Cephalexin	0.82 ± 0.48	36	73	91	100	
Cefaclor	0.93 ± 0.85	45	73	82	91	91
Cefazolin	0.82 ± 0.69	54	73	91	91	100
Cefamandole	0.56 ± 0.43	64	73	91	100	
Cefoxitin	0.82 ± 0.54	45	73	82	100	
Lactobacillus (11)						
Cephalothin	3.50 ± 0.80	18	27	54	91	91
Cephalexin	20.53 ± 0.95[c]	9	18	18	45	73
Cefaclor	17.02 ± 1.62	18	27	27	45	64
Cefazolin	1.86 ± 0.50	27	36	82	100	
Cefamandole	2.56 ± 0.65	27	36	45	91	100
Cefoxitin	16.00 ± 1.45	18	18	27	64	64
Bifidobacterium (11)						
Cephalothin	2.90 ± 0.14	27	27	54	91	100
Cephalexin	2.41 ± 0.64	18	45	67	91	100
Cefaclor	2.90 ± 0.95	27	45	54	73	73
Cefazolin	2.0 ± 0.79	18	54	64	91	100
Cefamandole	0.87 ± 0.55	45	64	82	100	
Cefoxitin	4.0 ± 0.76	9	45	54	73	100
Propionibacterium (85)						
Cephalothin	0.30 ± 0.21	83	95	100		
Cephalexin	0.91 ± 0.22[d]	8	81	99	100	
Cefaclor	2.78 ± 0.35[d]	3	22	73	94	100
Cefazolin	0.34 ± 0.20	62	99	100		
Cefamandole	0.31 ± 0.18	69	100			
Cefoxitin	0.34 ± 0.21	65	95	100		

[a] SD, Standard deviation.
[b] Significant difference compared with cephalothin; $P < 0.01$, t test analysis.
[c] Significant difference compared with cephalothin; $P < 0.05$, t test analysis.
[d] Significant difference compared with cephalothin; $P < 0.001$, t test analysis.

TABLE 3. *Comparative in vitro activity of cephalosporins against gram-negative anaerobic bacteria*

Organism tested against various agents (no. of isolates)	Geometric mean MIC ± SD (μg/ml)[a]	Cumulative % strains inhibited by (μg/ml):				
		≤0.25	1	4	16	64
Veillonella and *Acidaminococcus* (25)						
Cephalothin	0.54 ± 0.39	68	68	100		
Cephalexin	0.73 ± 0.35	32	76	96	100	
Cefaclor	0.35 ± 0.22	61	96	100		
Cefazolin	0.32 ± 0.21[b]	76	96	100		
Cefamandole	0.47 ± 0.39	72	80	96	100	
Cefoxitin	1.00 ± 0.36[b]	12	68	88	100	
Bacteroides fragilis (51)						
Cephalothin	128 ± 0.29				4	33
Cephalexin	55.71 ± 0.38[c]				29	61
Cefaclor	134.36 ± 0.32			2	4	23
Cefazolin	49.18 ± 0.34[c]				25	67
Cefamandole	97 ± 0.27			2	2	51
Cefoxitin	12.81 ± 0.29[c]			23	70	100
Bacteroides species (53)						
Cephalothin	5.09 ± 1.61	28	41	51	60	83
Cephalexin	2.82 ± 1.11	21	43	73	83	83
Cefaclor	4.00 ± 1.42	28	43	53	72	87
Cefazolin	2.94 ± 1.32	32	51	58	70	94
Cefamandole	2.98 ± 1.46	32	47	60	75	87
Cefoxitin	1.86 ± 0.75[d]	28	45	75	90	96
Fusobacterium (19)						
Cephalothin	0.80 ± 1.07	74	74	84	89	89
Cephalexin	1.19 ± 1.28	47	79	79	79	89
Cephaclor	2.00 ± 2.04	47	74	74	74	79
Cefazolin	0.74 ± 0.79	58	74	89	89	95
Cefamandole	0.77 ± 1.06	68	79	79	89	89
Cefoxitin	1.65 ± 1.34	37	68	68	84	89
Campylobacter (8)						
Cephalothin	4.75 ± 0.95	12	37	50	87	87
Cephalexin	17.38 ± 0.27				75	100
Cefaclor	11.31 ± 0.47			25	87	87
Cefazolin	13.45 ± 0.40			25	75	87
Cefamandole	7.31 ± 1.00		37	37	75	87
Cefoxitin	12.29 ± 1.37		37	37	37	75

[a] SD, Standard deviation.
[b] Significant differences compared with cephalothin; $P < 0.05$, t test analysis.
[c] Significant differences compared with cephalothin; $P < 0.001$, t test analysis.
[d] Significant differences compared with cephalothin; $P < 0.01$, t test analysis.

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