JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1988, p. 2361–2366
0095-1137/88/112361-06$02.00/0

Vol. 26, No. 11

Annual Incidence, Epidemiology, and Comparative In Vitro Susceptibilities to Cefoxitin, Cefotetan, Cefmetazole, and Ceftizoxime of Recent Community-Acquired Isolates of the *Bacteroides fragilis* Group

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The six species of the *Bacteroides fragilis* group are potent pathogens and commonly have different susceptibility patterns. We determined the relative annual isolation rate of anaerobic bacteria and the susceptibility of *B. fragilis* group species isolated during 1987 at two community hospitals. The relative frequencies of isolation of 261 strains were as follows: *B. fragilis*, 61%; *B. thetaiotaomicron*, 17%; *B. distasonis*, 7%; *B. vulgatus*, 6%; *B. ovatus*, 5%; and *B. uniformis*, 4%. A total of 234 recent clinical isolates were tested against cefmetazole, cefotetan, cefoxitin, ceftizoxime, clindamycin, imipenem, and piperacillin by a brucella agar dilution method. Imipenem was the most active agent tested with all but three isolates (two *B. vulgatus* and one *B. distasonis*) susceptible to <2 μg/ml. Of the cephalosporins tested, cefoxitin, cefotetan, and cefmetazole were relatively equal against *B. fragilis*, with 93 to 98% of strains susceptible to 32 μg/ml. Ceftizoxime was less active, with an MIC for 90% of strains tested of 128 μg/ml and only 75% of isolates susceptible to 32 μg/ml. Against *B. ovatus*, *B. vulgatus*, *B. thetaiotaomicron*, and *B. uniformis*, cefoxitin showed a two- to threefold-superior activity compared with that of cefotetan and cefmetazole. In general, ceftizoxime was much less active, except against *B. distasonis*, for which 78% of isolates were susceptible to 32 μg/ml compared with 68% for cefoxitin, 19% for cefmetazole, and 16% for cefotetan. Clindamycin and piperacillin showed activity similar to that of cefoxitin, except piperacillin was less active versus *B. vulgatus* and *B. distasonis*. We therefore suggest that clinical laboratories determine the species of *B. fragilis* group isolates as well as perform susceptibility studies on these isolates. Clinicians should be aware that while *B. fragilis* is the most frequent isolate, 38% of isolates are from other, more resistant *B. fragilis* group species.

Bacteroides fragilis is a well-known clinical pathogen that may be resistant to a variety of antimicrobial agents. In a nationwide survey, Tally et al. (24) noted regional differences in the susceptibility patterns of anaerobic bacteria and clustering of resistance at various hospitals. They therefore recommended that susceptibility studies should be performed on selected clinical isolates, especially of the *B. fragilis* group species, to monitor for outbreaks of infections caused by resistant organisms.

The *B. fragilis* group is composed of at least six bile-resistant species (*B. fragilis*, *B. distasonis*, *B. vulgatus*, *B. ovatus*, *B. thetaiotaomicron*, and *B. uniformis*) and contains other *B. fragilis* group organisms whose species have not yet been determined (16). The six species commonly have different susceptibility patterns (2, 26, 27). Despite this variability, most clinical microbiology laboratories are not identifying *B. fragilis* group isolates to the species level. Many laboratories do not test anaerobic bacteria for antimicrobial agent susceptibility. Other laboratories perform susceptibility studies but use a broth-disk elution test with only one clinically relevant antimicrobial agent concentration. While this may give grossly relevant clinical data, it may be inadequate for seriously ill patients for whom more specific information is important. Many of the *B. fragilis* group organisms have MICs that cluster at or near the breakpoint of the various antibiotics (22, 24, 26). A variety of alternative methods, including the National Committee for Clinical Laboratory Standards (NCCLS) reference method and microdilution methods, have become increasingly popular (5, 20–22). Adding to this complexity is the fact that numerous new antimicrobial agents with activity against anaerobic bacteria have become available.

Because of differences in patient populations and antimicrobial usage, it is difficult to extrapolate from the limited data available on the frequency of isolation of the various *B. fragilis* group species and the susceptibility of these individual species determined at foreign (6, 12, 14, 17, 19) and domestic (7, 15, 25, 27) research and tertiary care university hospitals to those situations encountered at community hospitals. Consequently, there are sparse data with which the many community clinicians may make rational therapeutic decisions.

In an effort to provide more information on pathogenic anaerobic organisms isolated at the community hospital, we collected all anaerobic bacterial isolates, except propionibacteria, and identified all *B. fragilis* group species isolated from clinical specimens at two community hospitals during 1987. We then compared the activities of cefoxitin, cefotetan, ceftizoxime, cefmetazole, clindamycin, imipenem, and piperacillin against 234 representative strains of the *B. fragilis* group.

MATERIALS AND METHODS

All anaerobic bacteria were isolated and identified according to standard criteria (16, 22). Data to determine the relative frequency of isolation of anaerobic bacteria were obtained from St. John's Hospital and Health Center (Santa Monica, Calif.) from January to December 1987.

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B. fragilis group species were identified by using prereduced anaerobically sterilized biochemicals (Carr Scarborough, Stone Mountain, Ga.). A total of 157 of the 234 isolates tested (67%) were obtained during the same time period from Santa Monica Hospital Medical Center and St. John's Hospital and Health Center, two primary care community hospitals in Santa Monica, Calif. The remaining 77 isolates were *B. fragilis* group species obtained from the same hospitals in 1984 and 1985 and utilized for comparison to detect the development of any drug resistance.

Standard laboratory powders were supplied as follows: cefotetan from Stuart Pharmaceuticals (Wilmington, Del.); ceftizoxime from Smith Kline & French Laboratories (Philadelphia, Pa.); cefoxitin and imipenem from Merck Sharp & Dohme (West Point, Pa.); piperacillin from Lederle Laboratories (Pearl River, N.Y.); cefmetazole and clindamycin from The Upjohn Co. (Kalamazoo, Mich.).

Strains were taken from frozen stock culture and transferred twice onto PRAS brucella agar (Anaerobe Systems, Santa Clara, Calif.) supplemented with 5% sheep blood, hemin, and vitamin K_1. Inocula were prepared by suspending colonies from overnight plates in brucella broth (Difco Laboratories, Detroit, Mich.) to achieve a 0.5 McFarland standard. The antimicrobial solutions were prepared on the day of study according to the instructions of the manufacturers. The Wadsworth agar dilution method was utilized (22). Brucella agar plates supplemented with 5% laked sheep blood, vitamin K_1, and hemin and the various antimicrobial agents to yield concentrations of 256 to 0.06 μg/ml were inoculated with a Steers replicator (Craft Machine Inc., Chester, Pa.). Control plates containing no antibiotic were inoculated before and after each set of antibiotic-containing plates. All plates were incubated for 48 h in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) or Anapak jars (Scott Laboratories, Fiskeville, R.I.). The MIC was defined as the lowest concentration of antimicrobial agent that allowed no growth, a barely visible haze, or one discrete colony. *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were included as controls in each test.

RESULTS

The relative frequencies of isolation of various anaerobic bacterial species and *B. fragilis* group species are shown in Tables 1 and 2, respectively. The *B. fragilis* group species accounted for 35% of all anaerobic isolates. Other frequent isolates included *Peptostreptococcus* species (29%), other *Bacteroides* species (11%), and *Fusobacterium* species (8%). Data from both hospitals revealed that of the *B. fragilis* group species, *B. fragilis* was the most frequently isolated (61%). *B. thetaiotaomicron* was also commonly isolated (17%). Table 3 compares our frequency of isolation of *B. fragilis* group species with isolations described in other recently published studies (4, 7, 14, 15, 22). Our results were similar to those of other United States studies (7, 15, 22). Recent surveys from Turkey (14) and India (4) had fewer *B. thetaiotaomicron* isolates and more frequent isolation of *B. ovatus*. This may reflect the similarity of these two organisms and the difficulty in separating these species. We used fermentation of xylan to separate *B. ovatus* from *B. thetaiotaomicron*.

Table 4 shows the relationship of the various species to the body site of isolation. *B. fragilis* was most often isolated from intra-abdominal infections (46%, 73 of 159). *B. fragilis* and *B. thetaiotaomicron* were more likely to be associated with bacteremias than were other *B. fragilis* group species.

TABLE 1. Relative frequency of isolation of anaerobic bacteria[a] from clinical sources in 1987[b]

Organism	No.	%
Bacteroides fragilis group	141	34.6
B. fragilis	77	18.9
B. thetaiotaomicron	12	3.0
B. vulgatus	10	2.4
B. distasonis	10	2.4
B. ovatus	6	1.5
B. uniformis	3	0.7
Unidentified (died)	23	5.7
Bacteroides species		
Pigmented	26	6.4
Other	45	11.0
Fusobacterium species	32	7.9
Peptostreptococcus species	117	28.7
Clostridium species	9	2.2
Nonsporeforming gram-positive bacilli	20	4.9
Gram-negative cocci	15	3.7
Microaerophilic streptococci	2	0.5
Total	407	100.0

[a] Excluding propionibacteria.
[b] From St. John's Hospital and Health Center, Santa Monica, Calif.

B. distasonis, *B. uniformis*, and *B. vulgatus* were infrequently associated with bacteremia. Peptostreptococci were equally frequent isolates of head and neck, soft tissue (below the waist), intra-abdominal, and female genital tract infections. Bacteremias caused by peptostreptococci were not noted. Clostridia were most often isolated from intra-abdominal sources, but 20% (8 of 40) of isolates were from blood.

The results of the susceptibility study of the *B. fragilis* group species are summarized in Table 5, which shows the percentage of organisms susceptible at several possible breakpoints (24, 26).

Imipenem was the most active agent tested, with almost all isolates of all species susceptible to <2 μg/ml. Two exceptions were one strain of *B. distasonis* for which the MIC was 8 μg/ml and two strains of *B. vulgatus* for which the MIC was 4 μg/ml. Of the four cephalosporins tested, cefoxitin, cefotetan, and cefmetazole were relatively equal against *B. fragilis*, with MICs for 90% of strains tested (MIC_{90}s) of 32, 16, and 32 μg/ml, respectively, and 93 to 98% of isolates susceptible at a breakpoint of 32 μg/ml. Ceftizoxime was less active against *B. fragilis*, with an MIC_{90} of 128 μg/ml and only 75% of isolates susceptible at 32 μg/ml. Against *B. vulgatus*, *B. ovatus*, *B. thetaiotaomicron*, and *B. uniformis*, cefoxitin was two- to threefold more active than

TABLE 2. Relative frequency of isolation of *B. fragilis* group species from clinical isolates in 1987

Species	St. John's Hospital		Santa Monica Hospital		Total	
	No.	%	No.	%	No.	%
B. fragilis	77	65.2	82	57.3	159	60.9
B. thetaiotaomicron	12	10.2	31	21.7	43	16.5
B. distasonis	10	8.5	8	5.6	18	6.9
B. vulgatus	10	8.5	7	4.9	17	6.5
B. ovatus	6	5.1	7	4.9	13	5.0
B. uniformis	3	2.5	8	5.6	11	4.2

TABLE 3. Percent distribution of *B. fragilis* group species in clinical isolates

Organism	% Distribution in:					
	United States				India (Chandigarh), 1987 (4)	Turkey (Istanbul), 1987 (14)
	VA Wadsworth, 1973–1983 (22)	University of Southern California, 1984 (15)	Walter Reed, 1973–1985 (7)	Santa Monica, 1987[a]		
B. fragilis	45	57	63	61	61	69
B. thetaiotaomicron	20	20	14	16	8	8
B. distasonis	10	8	7	7	9	2
B. vulgatus	10	8	7	6	6	9
B. ovatus	5	5	7	5	16	9
Other	10	2	2	4	0	3
Total no. of isolates		267	1,261	261	434	140

[a] Current study.

cefotetan and cefmetazole. At a breakpoint of 32 μg/ml, 92% of *B. thetaiotaomicron* isolates were susceptible to cefoxitin compared with 29% to cefmetazole and only 24% to cefotetan. For *B. ovatus* isolates, 82% were susceptible to cefoxitin but only 15% to cefmetazole and 12% to cefotetan. For *B. uniformis* isolates, 78% were susceptible to cefoxitin compared with 58% to both cefotetan and cefmetazole. In general, ceftizoxime was less active, with 75% of *B. fragilis*, 82% of *B. vulgatus*, 46% of *B. thetaiotaomicron*, and 58% of *B. uniformis* strains susceptible at a breakpoint of 32 μg/ml. Ceftizoxime was more active against *B. distasonis* than the other cephalosporins, with 78% susceptible compared with 69% susceptible to cefoxitin, 19% to cefmetazole, and 16% to cefotetan. For the non-*B. fragilis* species, there was no difference in susceptibility patterns between the 1986 to 1987 isolates and the 1984 to 1985 isolates, indicating no development of new resistance patterns.

Clindamycin and piperacillin showed activity similar to that of cefoxitin, except that piperacillin was less active against *B. vulgatus* and *B. distasonis* strains.

DISCUSSION

Anaerobic bacteria may cause any type of human infection including sepsis, peritonitis, and abdominal, cutaneous, and pulmonary abscesses (22). Because of their ability to produce β-lactamases, inactivating many common antimicrobial agents, and their particular ability to produce abscesses, members of the *B. fragilis* group are considered the most important anaerobic pathogens (10). The designation of six separate species in the *B. fragilis* group has necessitated a revised look at the frequency of isolation, pathogenicity, and antimicrobial susceptibility of each species (7, 15, 26). Several studies have emanated from research institutions (7, 15, 26), whose patient populations are often markedly different from those encountered at community hospitals.

Brook (7) recently reported the results of a 12-year experience at two military hospitals. He noted that *Bacteroides* species accounted for 43% of all anaerobes isolated and that members of the *B. fragilis* group accounted for 44% of all *Bacteroides* species recovered. Similarly, *Bacteroides* species accounted for 52% of all anaerobic bacteria isolated at our two community hospitals (Table 1). The isolation rates of all anaerobic bacteria and the frequency of *B. fragilis* group isolations were similar for both of our community hospitals. *B. fragilis* was the most frequently isolated species and accounted for 19% of all anaerobes isolated. The other *B. fragilis* group species accounted for 16% of isolates, with *B. thetaiotaomicron* second in frequency. In accord with the

TABLE 4. Distribution of anaerobic bacteria by clinical specimen source in 1987[a]

Anaerobic bacteria	No. of isolates								
	Blood	Head and neck	Pleuropulmonary	Soft tissue		Intra-abdominal	Perirectal abscess	Female genital tract	Miscellaneous
				Above waist	Below waist				
B. fragilis	19	1	4	1	33	73	3	11	4
B. thetaiotaomicron	5	0	0	0	6	25	3	3	1
B. vulgatus	2	0	0	0	0	8	3	3	1
B. distasonis	1	0	0	1	2	9	2	2	1
B. ovatus	1	1	0	0	1	8	2	0	0
B. uniformis	1	0	0	0	3	3	1	3	1
B. fragilis group, not identified	0	0	0	1	1	14	5	4	2
Pigmented *Bacteroides* sp.	1	9	1	2	2	5	0	2	1
Other *Bacteroides* sp.	1	14	4	5	2	12	2	10	3
Fusobacterium sp.	2	7	2	4	2	14	1	0	1
Peptostreptococci	0	23	3	9	22	26	10	20	7
Clostridia	8	2	0	0	6	24	0	0	0
Nonsporeforming gram-positive bacilli	3	8	0	3	1	23	2	0	2
Gram-negative cocci	1	5	1	2	0	1	1	4	0

[a] Santa Monica Hospital Medical Center and St. John's Hospital and Health Center, Santa Monica, Calif.

TABLE 5. Susceptibility of *B. fragilis* group species to seven antimicrobial agents

Organism	Total no. of isolates (no. of isolates 1984–1985)	Drug	MIC (μg/ml)			% Susceptible at breakpoint[a]:		
			Range	50%	90%	1	2	3
B. fragilis	57 (0)	Cefmetazole	4–128	16	32	47 (8)	84 (16)	98 (32)
		Cefotetan	0.5–>256	8	16	80.7 (8)	93 (16)	93 (32)
		Cefoxitin	2–64	8	32	63 (8)	84 (16)	97 (32)
		Ceftizoxime	≤0.5–>256	16	128	39 (8)	49 (16)	75 (32)
		Clindamycin	≤0.25–>256	≤0.25	2.0	72 (0.5)	91 (4)	91 (8)
		Imipenem	0.06–1	0.125	0.25	100 (4)	100 (8)	
		Piperacillin	0.5–>256	8	128	86 (16)	88 (32)	90 (64)
B. vulgatus	29 (12)	Cefmetazole	2–128	16	32	21 (8)	84 (16)	93 (32)
		Cefotetan	2–>256	8	32	79 (8)	86 (16)	93 (32)
		Cefoxitin	2–128	8	16	86 (8)	90 (16)	93 (32)
		Ceftizoxime	0.5–256	4	128	72 (8)	83 (16)	83 (32)
		Clindamycin	≤0.25–>256	≤0.25	1.0	86 (0.5)	93 (4)	93 (8)
		Imipenem	0.03–4	0.25	1.0	100 (4)	100 (8)	
		Piperacillin	2–>256	8	>256	72 (16)	72 (32)	83 (64)
B. distasonis	32 (16)	Cefmetazole	16–>256	64	>256	0 (8)	6 (16)	19 (32)
		Cefotetan	8–>256	128	256	3 (8)	6 (16)	16 (32)
		Cefoxitin	8–128	32	64	13 (8)	28 (16)	69 (32)
		Ceftizoxime	0.5–256	4	256	63 (8)	66 (16)	78 (32)
		Clindamycin	≤0.25–>256	1.0	4.0	44 (0.5)	94 (4)	97 (8)
		Imipenem	0.125–8	1.0	2.0	97 (4)	100 (8)	
		Piperacillin	4–>256	8	>256	69 (16)	72 (32)	72 (64)
B. thetaiotaomicron	63 (22)	Cefmetazole	2–128	64	128	8 (8)	10 (16)	29 (32)
		Cefotetan	1–>256	64	128	8 (8)	11 (16)	24 (32)
		Cefoxitin	4–64	32	32	10 (8)	32 (16)	92 (32)
		Ceftizoxime	2–>256	64	128	6 (8)	25 (16)	46 (32)
		Clindamycin	≤0.25–>256	2.0	4.0	18 (0.5)	94 (4)	95 (8)
		Imipenem	0.06–1	0.25	0.5	100 (4)	100 (8)	
		Piperacillin	2–>256	32	64	30 (16)	79 (32)	94 (64)
B. ovatus	34 (21)	Cefmetazole	8–256	64	128	3 (8)	6 (16)	15 (32)
		Cefotetan	16–128	64	128	0 (8)	3 (16)	12 (32)
		Cefoxitin	2–64	32	64	9 (8)	29 (16)	82 (32)
		Ceftizoxime	4–>256	32	128	9 (8)	41 (16)	68 (32)
		Clindamycin	≤0.25–>256	1.0	4.0	41 (0.5)	91 (4)	94 (8)
		Imipenem	0.125–2	0.25	0.5	100 (4)	100 (8)	
		Piperacillin	8–>256	32	64	47 (16)	85 (32)	91 (64)
B. uniformis	19 (6)	Cefmetazole	4–256	16	128	47 (8)	53 (16)	58 (32)
		Cefotetan	8–>256	32	128	16 (8)	42 (16)	58 (32)
		Cefoxitin	2–128	8	64	53 (8)	63 (16)	79 (32)
		Ceftizoxime	8–>256	32	>256	16 (8)	37 (16)	58 (32)
		Clindamycin	≤0.25–>256	1.0	>256	42 (0.5)	89 (4)	89 (8)
		Imipenem	0.125–1	0.25	0.5	100 (4)	100 (8)	
		Piperacillin	8–>256	16	256	53 (16)	74 (32)	79 (64)

[a] Number in parentheses is breakpoint.

reports of Brook (7) and Sutter et al. (22), we found that peptostreptococci were the second most frequently isolated group of anaerobic bacteria.

Intra-abdominal infection was the most common source for all anaerobic organisms and accounted for 42% of isolates. A total of 8% of isolates were from blood cultures. *B. fragilis* and *B. thetaiotaomicron* accounted for 53% of blood isolates, while *B. distasonis*, *B. ovatus*, *B. vulgatus*, and *B. uniformis* were infrequently isolated from blood. This is in accord with the results of Brook (7) and Heseltine et al. (15), who noted frequent isolation of *B. fragilis* and *B. thetaiotaomicron*, but rarely the other *B. fragilis* group species, from the blood. Brook (submitted for publication) reported that the mortality associated with bacteremia caused by *B. fragilis* (24%) and *B. ovatus* (20%) at Walter Reed Army Hospital was much lower than that for the bacteremia caused by *B. thetaiotaomicron* (38%), *B. vulgatus* (40%), and *B. distasonis* (50%). He speculated that the increased mortality might be related to the broader antimicrobial resistance pattern of those species, as was seen with our isolates (Table 5). In most series, including the current study, *Clostridium* species were also frequent blood isolates.

Community hospital clinical laboratories may not always isolate and identify all strains in multibacterial infections. However, the relative frequency of isolation of each member of the *B. fragilis* group species was similar to those reported from American university and research hospitals (Table 3) (7, 15, 22). One study from Turkey (14) and one from India (4) had results that were generally similar to those of American studies except for a lower frequency of *B. thetaiotaomicron* and a higher rate for *B. ovatus* isolation. Again, this might be due to differences in media, isolation, and

identification techniques used, or it could be due to differences in diet or the demographics of the populations studied.

Imipenem was the only agent tested with consistently good activity against virtually all isolates from all species of the *B. fragilis* group. Other studies (9, 17, 25) have also noted the excellent activity of imipenem against almost all isolates of all *B. fragilis* group species.

B. fragilis and *B. vulgatus* were generally equally susceptible to all other agents tested with the exception of ceftizoxime. A total of 25% of *B. fragilis* and 17% of *B. vulgatus* isolates were resistant to ceftizoxime at 32 μg/ml. *B. thetaiotaomicron* strains were generally resistant to cefmetazole, cefotetan, and ceftizoxime but susceptible to cefoxitin, clindamycin, and piperacillin. A similar pattern was seen with *B. ovatus* and *B. uniformis* strains. *B. distasonis* strains were also generally resistant to cefotetan and cefmetazole but susceptible to cefoxitin, clindamycin, and surprisingly, ceftizoxime. Cornick et al. (8) also noted cefmetazole to be less active than cefoxitin against *B. fragilis*, *B. thetaiotaomicron*, and *B. ovatus* isolates.

For a number of the strains tested, MICs were within one dilution of the breakpoint (clustering) for many of the agents tested. Table 5 shows the percentage of isolates susceptible to the various antimicrobial agents at several breakpoints, to allow clinicians to note any possible alterations that might occur when less than maximum dosage is used for therapy or to take into account the variability potentially resulting from such clustering.

Regardless of the method used for testing, cefotetan appeared to be consistently less active than cefoxitin. Andrews and Greenwood (3) studied eight *B. fragilis* group isolates that hydrolyzed cefoxitin and noted that cefotetan was less active than cefoxitin since β-lactamase enzyme extracts degraded cefotetan more rapidly than cefoxitin. Our results with a supplemented brucella agar dilution method are in accord with those of Aldridge et al. (2), who used a microdilution method with Anaerobe Broth (Difco), a formulation of Wilkins-Chalgren agar minus the agar, and noted that cefoxitin had better activity than cefotetan against all *Bacteroides* species tested, including those of the *B. fragilis* group. O'Keefe et al. (18), using the NCCLS Wilkins-Chalgren agar dilution method, reported that cefotetan was equivalent to cefoxitin against *B. fragilis* species isolates, but less active against other *B. fragilis* group species.

Controversy surrounds the degree of in vitro activity of ceftizoxime against *B. fragilis* group species. The widely varying results have been attributed by Aldridge and Sanders (1) and Wexler and Finegold (26) to methodologic differences. Aldridge and Sanders (1) compared the activity of various beta-lactam agents and found "antibiotic and method-dependent" differences, with ceftizoxime showing the greatest number of variations and discrepancies in susceptibility interpretation among the cephalosporins tested. Using the NCCLS agar dilution method, O'Keefe et al. (18) noted ceftizoxime to have a lower geometric mean MIC than cefoxitin against *B. fragilis* group species. Aldridge and Sanders (1) reported that ceftizoxime was more active against *B. distasonis* than was cefoxitin. Drulak and Chow (11), using Wilkins-Chalgren agar and an agar dilution method, also noted ceftizoxime to be more active than cefoxitin against *B. distasonis*. However, the MIC_{90}s of cefoxitin and ceftizoxime were 16 and 32 μg/ml, respectively, against 31 strains of *B. fragilis*. This is in contrast to our data and to those of Sutter et al. (22) obtained by a supplemented brucella agar dilution technique which showed cefoxitin to be more active than ceftizoxime against all *B. fragilis* group species except *B. distasonis*.

While the NCCLS reference agar dilution method (21) is commonly used in research centers, there are problems. Wexler and Finegold (26) noted that "the NCCLS procedure does not adequately support the growth of a number of anaerobes." Sutter et al. (22) noted that "some microorganism-drug combinations characteristically yielded rather poorly defined end points." In the original collaborative evaluation (21), cefoxitin was tested and gave consistent results, but none of the newer cephalosporins were tested. In subsequent studies (23, 28), the control strain of *Clostridium perfringens* gave inconsistent results when exposed to cefoperazone, and no MIC could be recommended. The control strains of *B. thetaiotaomicron* and *C. perfringens* gave inconsistent results when exposed to moxalactam in microdilution procedures, and no MICs could be recommended. Aldridge and Sanders (1) noted larger variations between the reference agar dilution method and several different broth microdilution methods, with brucella broth showing the least variation. Prior data from our laboratory (13) showed that the use of supplemented brucella media, either agar or broth, gave consistent MIC results and MBCs that were within two dilutions of the MICs for 26 *B. fragilis* group strains tested with cefoxitin. Consequently, the extrapolation of the NCCLS Wilkins-Chalgren agar dilution method to new drugs must be validated for each control organism-new drug combination.

Clinical implications of our incidence and in vitro data support several conclusions. Considering that 33 to 45% of *B. fragilis* group isolates are *B. distasonis*, *B. thetaiotaomicron*, *B. vulgatus*, and *B. uniformis* isolates and considering their frequent resistance to cefotetan and cefmetazole, the empiric use of these agents in seriously ill patients in whom these species are potential pathogens is cause for concern. In our study, ceftizoxime was consistently less active than cefoxitin, clindamycin, and piperacillin against *B. fragilis* group species including *B. fragilis* and *B. thetaiotaomicron* and excepting *B. distasonis*. Its use in seriously ill patients should also be approached cautiously. In addition, clinical laboratories should be encouraged to identify *B. fragilis* group isolates and perform in vitro susceptibility tests to aid clinicians in selecting and monitoring appropriate antimicrobial therapy of serious anaerobic infections.

ACKNOWLEDGMENTS

We thank Alice E. Goldstein, Judee H. Knight, and Gregory Ferguson for various forms of assistance.

This study was supported in part by a grant from Merck Sharp & Dohme, West Point, Pa.

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