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Susceptibility of Anaerobic Bacteria to Nine Antimicrobial Agents and Demonstration of Decreased Susceptibility of *Clostridium perfringens* to Penicillin

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The activity of moxalactam, cefoxitin, cephalothin, cefamandole, chloramphenicol, clindamycin, metronidazole, and ticarcillin was determined against 344 isolates of anaerobic bacteria. The activity of penicillin G was determined as well for 234 isolates not of the *Bacteroides fragilis* group. Moxalactam was more active than cephalothin and cefamandole and slightly less active than cefoxitin. Metronidazole was the most active antimicrobial agent against the *B. fragilis* group, whereas chloramphenicol was most active overall. *Clostridium* species were the most resistant group of organisms tested. Relatively high concentrations of penicillin were required to inhibit the *C. perfringens* strains: 80% at 0.5 U/ml and 100% at 16 U/ml. Our study demonstrates the need for periodic anaerobe susceptibility testing in order to better guide empiric antibiotic therapy.

Moxalactam (LY127935; compound 6059S) is a new semisynthetic β-lactam antibacterial agent highly active against a broad spectrum of microorganisms (11). Preliminary data suggest that it is active against *Bacteroides fragilis* and has various degrees of activity against other anaerobic bacteria (3, 11).

In this study we determined the activity of moxalactam against 344 strains of anaerobic bacteria and compared its activity with that of cefoxitin, cephalothin, cefamandole, chloramphenicol, clindamycin, metronidazole, penicillin, and ticarcillin.

Routine susceptibility testing of anaerobes is of limited usefulness in the immediate treatment of patients with anaerobic infections because of the delay in obtaining results. Such information is generally used only in treating patients with chronic anaerobic infections (4). Thus, empiric treatment is necessary for the majority of patients with anaerobic infections. In view of the changing susceptibility of anaerobes (2, 17) and the demonstration of plasmids in *B. fragilis*, *Bacteroides ochraceus*, and *Clostridium perfringens* (22), periodic determination of the susceptibility of a collection of recent isolates of anaerobes is necessary.

MATERIALS AND METHODS

Bacteria. All of the bacteria tested were recent clinical isolates from anaerobic specimens submitted to the Diagnostic Microbiology Laboratory of The Victoria General Hospital. Most of these isolates were obtained during the period August 1979 to April 1980. Organisms were identified according to procedures recommended by Holdeman and Moore (14). Species of approximately one-half of the *B. fragilis* group isolates were identified by using LD Presumpto plates (9). No attempt was made to identify the species of anaerobic gram-positive cocci.

The following control organisms, obtained from the American Type Culture Collection, were included with each run: *B. fragilis* ATCC 25285, *C. perfringens* ATCC 13124, and *Peptococcus magnus* ATCC 29328.

Media and susceptibility tests. Organisms to be tested were inoculated into PRAS brain heart infusion broth (Carr Scarborough, Atlanta, Ga.) and incubated at 35°C for 48 h. Turbidity was adjusted with brain heart infusion broth to that of one-half the no. 1 McFarland standard. An agar dilution susceptibility test was performed, using Wilkins-Chalgren agar (30). A Steers replicator was used to inoculate the suspension to agar surfaces (23). At the beginning and end of each series of tests, two plates of test medium without antibiotics were inoculated. One was incubated anaerobically to serve as a growth control, and the other was incubated aerobically to detect possible aerobic contamination. An anaerobic growth control plate was done between each set of the different antibiotics. Anaerobic incubation was carried out for 48 h at 35°C in an anaerobic chamber (Forma Scientific, Marietta, Ohio), containing a gas mixture of 90% nitrogen, 5% carbon dioxide, and 5% hydrogen.

The minimal inhibitory concentration (MIC) was read as the lowest concentration of antimicrobial agent yielding no growth, up to three discrete colonies, or a barely visible haze.

Antimicrobial agents. Laboratory standard powders were supplied as follows: penicillin G, cephalothin, cefamandole, and moxalactam from Eli Lilly & Co. Ltd., Toronto, Ontario; ticarcillin from Beecham Lab-

oratories, Pointe Claire, Quebec; chloramphenicol from Parke, Davis & Co., Brockville, Ontario; metronidazole from Poulenc, Montreal, Quebec; clindamycin from The Upjohn Co., Don Mills, Ontario; and cefoxitin from Charles E. Frosst, Montreal, Quebec.

RESULTS AND DISCUSSION

Control strains. MICs for test strains were accepted only if the MICs for the control strains (*B. fragilis* ATCC 25285, *C. perfringens* ATCC 13124, and *P. magnus* ATCC 29328) were within the range given by Sutter and Finegold (26) for penicillin G, clindamycin, chloramphenicol, and cefoxitin. Overall, 70% of the MICs were on the mode MIC.

Moxalactam. The activity of moxalactam and the other agents tested is shown in Tables 1 and 2. Generally the concentration required to inhibit 90% of *B. fragilis* group isolates was higher than in previous studies. Fass found that 90% of 15 strains of *B. fragilis* were inhibited by 2 μg/ml (11), whereas Borobio et al. found that 90% of 30 such isolates were inhibited by 0.5 μg/ml (3). Similarly, Jorgensen et al. (15) found that moxalactam was very active against *B. fragilis*; 90% of 28 isolates were inhibited by 2 μg/ml. None of the 49 *B. fragilis* group isolates in that study required >64 μg/ml for inhibition of growth. In a recent study, Carter et al. (W. T. Carter, D. R. Pyeatt, and S. M. Finegold, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, A45, p. 8) found that 16 μg/ml was required to inhibit all 28 isolates of *B. fragilis*.

All 16 isolates of *Bacteroides vulgatus* in our study were inhibited by ≤32 μg/ml compared

TABLE 1. *Comparative activities of eight antibiotics against B. fragilis group isolates*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		50%	90%	Range
B. fragilis (56)	Moxalactam	4	64	0.125->128
	Cephalothin	>128	>128	16->128
	Cefamandole	64	>128	2-128
	Cefoxitin	8	16	0.25-64
	Ticarcillin	32	>256	0.5->256
	Metronidazole	0.5	2	0.125-4
	Clindamycin	<0.25	1	0.25->128
	Chloramphenicol	4	4	2-16
B. distasonis (29)	Moxalactam	4	64	0.25->128
	Cephalothin	>128	>128	4->128
	Cefamandole	64	128	8-128
	Cefoxitin	8	64	2-64
	Ticarcillin	32	256	0.5-256
	Metronidazole	0.25	1	0.125-2
	Clindamycin	0.25	2	0.25-2
	Chloramphenicol	4	16	2-16
B. vulgatus (16)	Moxalactam	2	32	0.5-32
	Cephalothin	64	>128	32->128
	Cefamandole	64	>128	16->128
	Cefoxitin	8	32	2-32
	Ticarcillin	16	64	0.5->256
	Metronidazole	0.25	1	0.125-2
	Clindamycin	0.25	1	0.25-2
	Chloramphenicol	4	8	4-16
B. thetaiotaomicron (7)	Moxalactam	16	>128	1->128
	Cephalothin	>128	>128	64->128
	Cefamandole	64	>128	64->128
	Cefoxitin	16	64	0.5-64
	Ticarcillin	32	>256	1->256
	Metronidazole	0.5	0.5	0.5
	Clindamycin	0.5	2	0.5-2
	Chloramphenicol	8	16	4-16
B. ovatus (2)	Moxalactam	1	4	1-4
	Cephalothin	16	>128	16->128
	Cefamandole	64	>128	64->128
	Cefoxitin	4	4	4
	Ticarcillin	8	64	8-64
	Metronidazole	0.25	0.5	0.25-0.5
	Clindamycin	0.5	0.5	0.5
	Chloramphenicol	4	4	4

TABLE 2. *Comparative activities of nine antibiotics against various anaerobic bacteria*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		50%	90%	Range
B. melaninogenicus (15)	Moxalactam	0.125	4	0.06–8
	Cephalothin	1	32	0.25–32
	Cefamandole	1	32	0.25–64
	Cefoxitin	0.5	16	0.25–16
	Penicillin G	0.25	4	0.25–8
	Ticarcillin	0.5	8	0.5–8
	Metronidazole	0.25	1	0.125–2
	Clindamycin	0.25	0.5	0.25–2
	Chloramphenicol	1	4	0.25–8
Other *Bacteroides* (80)	Moxalactam	1	8	0.06–>128
	Cephalothin	32	>128	0.25–>128
	Cefamandole	8	>128	0.25–>128
	Cefoxitin	2	32	0.25–>128
	Penicillin G	2	16	0.25–>128
	Ticarcillin	8	64	0.25–128
	Metronidazole	0.25	4	0.25–>128
	Clindamycin	0.25	0.5	0.25–8
	Chloramphenicol	2	8	0.25–8
Fusobacterium spp. (17)	Moxalactam	0.125	4	0.06–>128
	Cephalothin	0.25	>128	0.25–>128
	Cefamandole	0.25	64	0.25–>128
	Cefoxitin	0.25	>128	0.25–>128
	Penicillin G	0.25	2	0.25–4
	Ticarcillin	0.5	16	0.25–>256
	Metronidazole	0.125	0.5	0.125–2
	Clindamycin	0.25	0.5	0.25–1
	Chloramphenicol	0.5	1	0.25–4
Anaerobic gram-positive cocci (60)	Moxalactam	0.125	32	0.06–>128
	Cephalothin	0.25	32	0.25–>128
	Cefamandole	0.25	32	0.25–64
	Cefoxitin	0.25	8	0.25–32
	Penicillin G	0.25	2	0.25–>128
	Ticarcillin	0.5	1	0.25–128
	Metronidazole	0125	0.5	0.125–>128
	Clindamycin	0.25	4	0.25–32
	Chloramphenicol	1	4	0.25–8
C. perfringens (45)	Moxalactam	0.125	32	0.06–>128
	Cephalothin	1	32	0.25–64
	Cefamandole	1	>128	0.25–>128
	Cefoxitin	0.5	8	0.25–64
	Penicillin G	0.25	8	0.25–16
	Ticarcillin	0.5	2	0.5–128
	Metronidazole	0.5	1	0.125–1
	Clindamycin	0.25	2	0.25–8
	Chloramphenicol	2	4	1–4
Other *Clostridium* species (17)	Moxalactam	8	>128	0.125–>128
	Cephalothin	2	32	0.5–>128
	Cefamandole	1	16	0.25–>128
	Cefoxitin	4	>128	0.25–>128
	Penicillin G	0.25	>128	0.25–>128
	Ticarcillin	1	128	0.5–128
	Metronidazole	0.25	>128	0.125–>128
	Clindamycin	1	>128	0.25–>128
	Chloramphenicol	2	4	1–4

[a] MIC for penicillin G is given as units per milliliter.

with 89% of *B. fragilis*, 82% of *Bacteroides distasonis*, and 85% of *Bacteroides thetaiotaomicron* isolates. *Bacteroides melaninogenicus* was the most susceptible *Bacteroides* species to this antibiotic, with all 15 isolates inhibited by 8 μg/ml. Ninety percent of all other anaerobes tested, with the exception of *Clostridium* species, were inhibited by ≤32 μg/ml. Only 70% of the *Clos-*

tridium species were inhibited by 32 μg/ml. Various organisms within this group have been shown to be more resistant to clindamycin than the majority of other anaerobes (7, 31), and in this study we show that this group of organisms is also more resistant to moxalactam, cefoxitin, metronidazole, and clindamycin. No strains of *Clostridium difficile* were included.

Cephalosporins and similar drugs. Generally cephalothin and cefamandole were inactive against the majority of *B. fragilis* group strains at 32 μg/ml, a finding in keeping with other studies (1, 6, 7). Cefoxitin was most active, inhibiting 90% of *B. fragilis*, *B. distasonis*, *B. vulgatus*, and *Bacteroides ovatus* at ≤32 μg/ml. Such activity is due to the resistance of cefoxitin, a cephamycin derivative, to the β-lactamase of *B. fragilis* (18, 28). Both cefamandole and cephalothin were more active than cefoxitin against *Clostridium* species other than *C. perfringens*. A similar finding was also noted by Sutter and Finegold (25) for cephalothin and cefoxitin.

Penicillins. *B. fragilis* group strains were not tested against penicillin G. Most of the 234 anaerobes tested were inhibited by ≤32 U of penicillin G per ml. This level can be achieved in the serum if a high parenteral dosage is given (26). A surprising finding in this study was the decreased activity of this antibiotic against *C. perfringens* in comparison with findings of other studies. All of our 45 strains were inhibited by 16 U/ml. In contrast, in another study from Canada, Dubois et al. (10) found that 0.5 μg/ml inhibited 22 strains of *Clostridium*. In another study, 95% of 238 strains of *C. perfringens* were susceptible to 0.25 μg/ml (7). Decreased activity of penicillin G against *Clostridium* species was also noted in our study. Whether this finding represents a trend remains to be substantiated, but data of Brown and Waatti suggest that this is so (5). Eighty-nine percent of their 44 isolates of *C. perfringens* were inhibited by penicillin G at 4 μg/ml, and 86% of *Clostridium innocuum* were inhibited by 8 μg/ml. Relatively high MICs of penicillin for *Clostridium clostriiforme*, *C. difficile*, *Clostridium paraputrificum*, and *Clostridium ramosum* were also noted by these investigators.

Earlier studies suggested that nearly all *Bacteroides* isolates were inhibited by ≤128 μg of ticarcillin per ml (20, 29). In a more recent study, only 88% of 25 *B. fragilis* were inhibited by 100 μg/ml (16). This latter study correlates with our data in that only 68% of our 53 *B. fragilis* strains were inhibited by ≤128 μg/ml.

Metronidazole. Metronidazole was the most active agent tested against the *B. fragilis* group of organisms. All were inhibited by ≤4 μg/ml. Resistance to metronidazole has previously been seen only among the microaerophilic and anaerobic streptococci, *Actinomyces*, and *Propionibacterium* (12). Two recent studies have reported some strains of *B. melaninogenicus* and other *Bacteroides* resistant to metronidazole (1, 17). Three of our 18 strains (17%) of *Clostridium*, other than *C. perfringens*, were resistant to metronidazole. In vitro metronidazole can be inactivated by aerobic bacteria; however, the clinical significance of this finding remains to be determined (19).

Clindamycin. Clindamycin was active against most of the isolates at ≤8 μg/ml. One of our 56 *B. fragilis* strains was resistant to clindamycin. Such resistance remains unusual even after widespread use of clindamycin (1, 8, 26). Clindamycin-resistant strains of *B. ovatus* have been isolated from two bacteremic patients (21) and, using a broth method, Bawdon et al. (2) found that 11 of 92 isolates of *B. fragilis* in four Detroit area hospitals had a clindamycin MIC of ≥8 μg/ml, and six of these isolates required 256 to 512 μg of clindamycin for inhibition of growth. Hansen (13) has noted that *B. thetaiotaomicron* and *B. distasonis* have higher MICs for clindamycin than other organisms within the *B. fragilis* group. Resistance to clindamycin was found in our study among the anaerobic gram-positive cocci and *Clostridium* species, a finding consistent with previous data (24).

Chloramphenicol. A chloramphenicol serum level of 16 μg/ml is readily achievable, and all 342 strains tested were inhibited at this concentration. Such susceptibility, however, is not always translated into therapeutic success. Thadepalli et al. (29) reported 10 patients with severe anaerobic infections who failed to respond to intravenous chloramphenicol. They were subsequently cured with clindamycin.

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