ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1990, p. 671–673
0066-4804/90/040671-03$02.00/0

Vol. 34, No. 4

Susceptibilities of Species of the *Bacteroides fragilis* Group to 10 Antimicrobial Agents

CARMEN BETRIU,* ESTHER CAMPOS, CARMEN CABRONERO, CARMEN RODRIGUEZ-AVIAL, AND JUAN J. PICAZO

Servicio de Microbiología Clínica, Hospital Universitario San Carlos, 28040 Madrid, Spain

Received 10 August 1989/Accepted 8 January 1990

A total of 94 clinical isolates of the *Bacteroides fragilis* group was tested for susceptibility to metronidazole, chloramphenicol, clindamycin, cefoxitin, cefotetan, cefmetazole, moxalactam, mezlocillin, amoxicillin-clavulanic acid, and imipenem. All the strains tested were susceptible to imipenem, metronidazole, amoxicillin-clavulanic acid, and chloramphenicol. The rate of resistance to clindamycin was 21%. The results of this study demonstrate a difference in resistance rates from one species of the *B. fragilis* group to another.

During the last few years, anaerobic bacteria have shown an increase in resistance to most of the traditionally used antimicrobial agents and some of the newer beta-lactam agents (8, 12, 14, 24). The anaerobic bacteria most frequently isolated from clinical infections are members of *Bacteroides fragilis* group.

Recent reports (2, 3, 7, 11, 26) have emphasized the changing susceptibility patterns of *Bacteroides* species and the difference in resistance rates to antibiotics displayed by the different species of the *B. fragilis* group.

We determined the MICs of some of the newer antimicrobial agents against the *B. fragilis* group; these were compared with the MICs of other antimicrobial agents frequently used in anaerobic infections. A comparison of the different susceptibility patterns displayed by the various species of the *B. fragilis* group was also made.

A total of 94 clinical isolates of the *B. fragilis* group was tested: 58 *B. fragilis*, 16 *B. thetaiotaomicron*, 10 *B. ovatus*, 6 *B. distasonis*, and 4 *B. vulgatus* isolates. Species identification was carried out with the AN Ident system (Bio Mérieux).

Antibiotics were kindly provided as follows: metronidazole, Rhône-Poulenc Farma, S.A.E.; chloramphenicol and cefmetazole, Antibióticos, S.A.; clindamycin, Upjohn Farmoquímica, S.A.; cefoxitin and imipenem, Merck Sharp & Dohme; cefotetan, ICI-Farma, S.A.; moxalactam, Eli Lilly & Co., S.A.; mezlocillin, Química Farmacéutica Bayer, S.A.; and amoxicillin-clavulanic acid (2:1), Laboratorios Beecham, S.A.

Antimicrobial susceptibility tests were performed by the National Committee for Clinical Laboratory Standards reference agar dilution method (18) with Wilkins-Chalgren agar (Oxoid Ltd.). Antibiotic dilutions ranged from 256 to 0.125 μg/ml, except for imipenem, which was tested at 32 to 0.016 μg/ml. The agar dilution test plates were inoculated with a Steers replicator and incubated at 35°C for 48 h in an anaerobic chamber with inocula of approximately 10^5 CFU.

The MIC was defined as the lowest concentration of an antimicrobial agent that yielded no growth, one discrete colony, or a fine, barely visible haze as determined with the unaided eye. MICs were determined for the group as a whole as well as for individual species. β-Lactamase activity was tested with a chromogenic cephalosporin substrate, nitrocefin (4).

* Corresponding author.

The results of the in vitro study with 94 strains of the *B. fragilis* group are summarized in Tables 1 and 2. β-Lactamase production was detected in 95% of strains.

All the strains tested were susceptible to metronidazole and chloramphenicol. Imipenem and amoxicillin-clavulanic acid were the most active beta-lactam drugs and had similar activities against all species of the *B. fragilis* group. No resistance was found.

Mezlocillin showed good activity with low resistance rates for the entire group (3%) as well as for the individual species. The inhibitory activity of mezlocillin was found to be lowest against *B. ovatus*, with a resistance rate of 10%.

The resistance rate of the *B. fragilis* group to clindamycin was 21%. Clindamycin-resistant strains occurred at rates of 50, 30, and 33% in *B. thetaiotaomicron*, *B. ovatus*, and *B. distasonis*, respectively, whereas in *B. fragilis* the resistance rate was lower (12%) (Table 2). All the *B. vulgatus* strains tested were susceptible to clindamycin.

The activities of cefoxitin, cefotetan, and moxalactam were comparable for the *B. fragilis* group, with MICs for 90% of the strains of 16, 32, and 16 μg/ml, respectively. Cefmetazole was less active; the MIC for 90% of the strains was 64 μg/ml. As reported previously (3, 8, 16, 26), members of the group differed in their susceptibilities to the four cephamycins tested. Cefmetazole exhibited the highest variation in activity from one species to another: the lowest cefmetazole resistance rate (15.5%) was found in *B. fragilis*, and the highest (70%) was found in *B. ovatus*. Moxalactam showed less species variation in activity and was more active than cefmetazole, cefoxitin, and cefotetan against *B. thetaiotaomicron*. Cefotetan showed good activity against *B. fragilis* and lesser activity against *B. thetaiotaomicron*, *B. ovatus*, and *B. distasonis*. This results are comparable to those of Werner (25) and Edmiston et al. (13). Cefmetazole displayed poor activity for the non-*B. fragilis* species included in the *B. fragilis* group.

B. fragilis tended to be much more susceptible to the cephamycins than the other members of the group (Table 2).

The results of this study indicate that several of the new beta-lactam agents are effective in vitro against the *B. fragilis* group. As expected, chloramphenicol and metronidazole were uniformly effective against all 94 isolates. Imipenem was the most effective beta-lactam drug in this study; the MIC for 90% of the strains was 0.5 μg/ml, and we detected no resistance, which was in accordance with the

TABLE 1. Antimicrobial agents against the *B. fragilis* group

Antimicrobial agent	MIC (μg/ml)[a]			% Resistant strains at low (high) breakpoint	Low (high) breakpoint for resistance (μg/ml)
	Range	50%	90%		
Metronidazole	0.125–4	0.5	0.5	0 (0)	8 (16)
Chloramphenicol	1–16	2	8	0 (0)	8 (16)
Clindamycin	≤0.06–>256	1	>256	21 (21)	4 (8)
Cefoxitin	2–128	8	16	22 (6)	16 (32)
Cefotetan	1–128	8	32	26 (9)	16 (32)
Cefmetazole	1–128	16	64	58 (32)	16 (32)
Moxalactam	0.125–>256	4	16	10 (6)	16 (32)
Mezlocillin	1–>256	8	32	5 (3)	64 (128)
Amoxicillin-clavulanic acid (2:1)[b]	0.25–8	0.5	2	0 (0)	8 (16)
Imipenem	≤0.06–2	0.125	0.5	0 (0)	4 (8)

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.
[b] For amoxicillin plus clavulanic acid, MICs are given as the concentration of amoxicillin.

literature, in which only occasional resistant strains of *B. fragilis* have been reported (6, 17).

The combination of clavulanic acid and amoxicillin showed good activity against *B. fragilis* and other *B. fragilis* group strains. Our results are similar to the data reported by others (3, 5, 15).

The incidence of clindamycin resistance in the *B. fragilis* group has been reported by several investigators (1, 8, 20, 22), although there is considerable variation in the resistance rates observed in different surveys, ranging from very low (0.6%, Bourgault et al. [2]) to moderate (7%, Tally et al. [24], and 10%, Derriennic et al. [10]) to high resistance rates similar to ours (about 20% clindamycin resistance [1, 9, 19, 21]).

This study confirms the reports of other investigators (3, 8, 16, 26) regarding the variation of susceptibility patterns among the species of the *B. fragilis* group, particularly with susceptibility to the beta-lactam agents. Souza Dias et al. (23) observed that the high resistance level was not limited to indole-positive strains, as suggested by Jenkins et al. (16), but was also present in *B. distasonis*. Of the various species in the group, *B. fragilis* is the most susceptible to the cephamycins; the species with lowest susceptibility to cephamycins were *B. thetaiotaomicron*, *B. ovatus*, and *B. distasonis*.

These data emphasize the need to identify species in the *B. fragilis* group and to determine the susceptibility patterns in order to facilitate the selection of adequate antimicrobial therapy.

Because of the increasing resistance of anaerobic bacteria to antimicrobial agents and because of regional differences in resistance patterns (1, 2, 7, 11, 24), we conclude that clinical microbiology laboratories should perform periodic susceptibility studies on anaerobic bacteria in order to detect changes in susceptibility profiles, to determine patterns of susceptibility of anaerobes to new antimicrobial agents, and to guide the choice of agents for the treatment of infections caused by anaerobic bacteria.

TABLE 2. Resistance rates of *B. fragilis* group species

Species (no. of isolates)	% Resistant to[a]:					
	Clindamycin	Cefoxitin	Cefmetazole	Moxalactam	Cefotetan	Mezlocillin
All *B. fragilis* group species (94)	21	6	32	6	9	3
B. fragilis (58)	12	3	15	5	3	1
B. thetaiotaomicron (16)	50	12	56	6	25	6
B. ovatus (10)	30	10	70	10	20	10
B. distasonis (6)	33	16	66	16	16	0
B. vulgatus (4)	0	0	25	0	0	0

[a] Resistance breakpoints were the high breakpoints from Table 1. No resistance to imipenem or amoxicillin-clavulanic acid was found.

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