

Susceptibility to Five Antimicrobial Agents of Strains of the *Bacteroides fragilis* Group Isolated in Brazil

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The in vitro activity of metronidazole, chloramphenicol, clindamycin, cefoxitin, and carbenicillin was tested by an agar dilution method against 228 strains of the *Bacteroides fragilis* group isolated from human intestinal microbiota during 1981 and 1982. All the strains were susceptible to metronidazole. Resistance rates for chloramphenicol, clindamycin, cefoxitin, and carbenicillin were 2, 37, 21, and 13%, respectively.

Anaerobic bacteriology is not a routine method for the laboratory diagnosis of infectious diseases in Brazil. The isolation and identification of anaerobic bacteria from clinical material and determination of their susceptibility to antimicrobial agents is restricted to a few university hospitals and research institutes (3). In most cases, treatment of clinically suspected anaerobic infections is decided on the basis of data found in the international scientific literature. Unfortunately, we do not have a significant number of clinical isolates of these microorganisms to evaluate their susceptibility to antimicrobial agents. Considering the prevalence of the *Bacteroides fragilis* group in these anaerobic infections, their endogenous origin, and the possibility that regional differences occur among resistant strains (11), we studied the susceptibility to five antimicrobial agents of bacteria isolated from human intestinal microbiota. Metronidazole, chloramphenicol, clindamycin, cefoxitin, and carbenicillin were selected because they are usually recommended for the treatment of anaerobic infections in Brazil.

Microorganisms were cultivated from fecal samples obtained from 60 individuals between the ages of 1 month and 65 years during 1981 and 1982. Forty individuals had no antimicrobial therapy for at least 1 month before the sampling, whereas the other twenty were patients with antimicrobial usage during the period of sampling and 1 week before. Antimicrobial agents used in the treatment of these patients were aminoglycosides, penicillins, cephalosporins, erythromycin, tetracycline, chloramphenicol, and metronidazole. A total of 228 *B. fragilis* group strains was isolated by using selective *B. fragilis* bile-esculin (BBE) medium (6) incubated anaerobically in jars containing a gaseous mixture (80% N₂, 10% CO₂, 10% H₂) at 37°C for 48 h. Four colonies were picked from each sample and identified to the species level. *B. fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides vulgatus*, and *Bacteroides uniformis* were characterized biochemically (10). MICs of the antimicrobial agents were determined by an agar dilution method with a Steers replicator (12). The medium used was brain heart infusion agar supplemented with defibrinated sheep blood (50 ml/liter), yeast extract (5 g/liter), hemin (0.005 g/liter), and menadione (0.005 g/liter). Chloramphenicol (Sigma Chemical Co., St. Louis, Mo.) and clindamycin (The Upjohn Co., Kalamazoo, Mich.) were diluted into medium to concentrations of 0.5 to 512 µg/ml, cefoxitin (Merck Sharp & Dohme, Rahway, N.J.) and car-

benicillin (Pfizer Inc., New York, N.Y.) concentrations ranged from 2 to 512 µg/ml, and metronidazole (Rhodia S.A. Div. Farmacêutica, São Paulo, Brazil) concentrations ranged from 0.125 to 32 µg/ml, all in twofold serial dilutions. Inoculum was prepared from a brain heart infusion-supplemented broth diluted to a density of 10⁷ CFU/ml; the concentration of organisms deposited on the agar with the Steers replicator was approximately 3 × 10⁴ CFU/ml. Reference strains of *B. fragilis* (ATCC 25285) and *B. thetaiotaomicron* (ATCC 29741) were included in each experiment to assess the reliability of the method. Inoculated plates were incubated in anaerobic jars at 37°C for 48 h. After the incubation period, the MIC for each strain tested was recorded as the lowest concentration of antimicrobial agent that prevented macroscopic growth.

The susceptibility of the 228 bacterial strains to the five drugs are shown in Table 1 as the MICs for 50 and 90% of isolates tested (MIC₅₀ and MIC₉₀, respectively), the ranges of MICs observed, and the percentages of resistant bacteria. Resistance to metronidazole was not observed, and few strains showed resistance to chloramphenicol. As previously described, metronidazole resistance among the *B. fragilis* group is not common (13). Only one metronidazole-resistant strain was reported by Ingham et al. (5), who isolated the microorganism from the feces of a patient submitted to long-term therapy with the drug for Crohn's disease. However, a significant number of our bacteria showed resistance to clindamycin. The MICs for some strains reached levels higher than 512 µg/ml. The occurrence of resistance to clindamycin in the *B. fragilis* group has been reported by many others (2, 4, 7, 9, 11), and it is known that the clinical use of clindamycin or erythromycin may increase the frequency of clindamycin resistance among *Bacteroides* species, because the genes coding for this resistance also code for erythromycin resistance (13). The frequent prescription of erythromycin in Brazil could explain the high percentages of clindamycin-resistant strains we found, because we observed that half of the microorganisms tested were simultaneously resistant to both the drugs. Although cefoxitin has been considered resistant to bacterial beta-lactamase (1), in our study a significant number of strains showed resistance to this cephamycin. The MIC of cefoxitin reached 128 µg/ml, and the MIC₉₀ for the 228 strains tested was 64 µg/ml. Although results may vary with different laboratories and different studies, noteworthy is the fact that the MIC₉₀ of cefoxitin for isolates of the *B. fragilis* group in the United States during 1981 and 1982 was only 16 µg/ml (2). We also

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TABLE 1. Comparative in vitro activity of metronidazole, chloramphenicol, clindamycin, cefoxitin, and carbenicillin against species of the *B. fragilis* group

Organism (no. of isolates)	Antimicrobial agent	MIC ($\mu\text{g/ml}$)			% Resis- tant ^a
		Range	50%	90%	
<i>Bacteroides fragilis</i> (94)	Metronidazole	1-16	4	8	0
	Chloramphenicol	2-16	8	8	0
	Clindamycin	1->512	4	>512	42
	Cefoxitin	4-128	16	64	17
	Carbenicillin	8->512	64	>512	15
<i>Bacteroides vulgatus</i> (21)	Metronidazole	1-16	4	8	0
	Chloramphenicol	4-8	8	8	0
	Clindamycin	1->512	2	>512	19
	Cefoxitin	8-128	16	64	14
	Carbenicillin	4->512	32	64	5
<i>Bacteroides distasonis</i> (41)	Metronidazole	1-16	2	8	0
	Chloramphenicol	2-16	8	8	0
	Clindamycin	1->512	4	>512	39
	Cefoxitin	2-128	16	64	12
	Carbenicillin	8->512	32	>512	19
<i>Bacteroides ovatus</i> (25)	Metronidazole	4-8	2	8	0
	Chloramphenicol	4-32	8	32	12
	Clindamycin	1->512	2	>512	40
	Cefoxitin	8-128	32	64	28
	Carbenicillin	16->512	64	256	25
<i>Bacteroides uniformis</i> (17)	Metronidazole	2-8	4	4	0
	Chloramphenicol	2-16	8	16	0
	Clindamycin	2->512	8	512	59
	Cefoxitin	4-64	4	64	12
	Carbenicillin	32-128	32	64	0
<i>Bacteroides thetaiotaomicron</i> (30)	Metronidazole	1-8	4	8	0
	Chloramphenicol	4-32	8	16	3
	Clindamycin	1->512	2	16	17
	Cefoxitin	4-128	32	64	47
	Carbenicillin	32-512	128	128	7
All <i>Bacteroides fragilis</i> group (228)	Metronidazole	1-16	4	8	0
	Chloramphenicol	2-32	8	8	2
	Clindamycin	1->512	2	>512	37
	Cefoxitin	2-128	16	64	21
	Carbenicillin	4->512	64	512	13

^a Numbers are the percentages of resistant strains at breakpoints as follows: metronidazole, 16 $\mu\text{g/ml}$; chloramphenicol, 16 $\mu\text{g/ml}$; clindamycin, 4 $\mu\text{g/ml}$; cefoxitin, 32 $\mu\text{g/ml}$; carbenicillin, 128 $\mu\text{g/ml}$.

observed that some strains were resistant to the beta-lactam antibiotic carbenicillin. The occurrence of microorganisms resistant to this drug at levels higher than 512 $\mu\text{g/ml}$ could be explained by the presence of a new beta-lactamase in *B. fragilis* species, as reported by Sato et al. (8).

We believe that regional differences in resistance patterns that occur in the *B. fragilis* group (11) are an important

argument to motivate laboratories to start developing anaerobic bacteriology in Brazil. They also should stimulate the establishment of a reference center for the study of anaerobic bacteria, because there are already such centers for streptococci, members of the family *Enterobacteriaceae*, and *Corynebacterium diphtheriae*.

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