

Virulence Markers and Antimicrobial Susceptibility of Bacteria of the *Bacteroides fragilis* Group Isolated from Stool of Children with Diarrhea in São Paulo, Brazil

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Bacteroides fragilis has been isolated from several human and non-human monomicrobial and mixed infections. In this study, some virulence markers and the antimicrobial susceptibility of bacteria of the *B. fragilis* group isolated from children's stools were evaluated. All the 64 isolates showed the following characteristics: capsulated, β -hemolytic, hydrophilic, and serum-resistant. Only, 24 (37.5%) strains were resistant at 60°C, for 30 min, and among them, 12 (18.75%) were resistant at 60°C, for 60 min. Also, none strain was resistant at 100°C. Four strains were able to hemagglutinate erythrocytes and D-mannose, D-galactose, D-arabinose, and D-xylose inhibited hemagglutination in 2 *B. fragilis* strains (p76a, p76b). The hemagglutination in the strain *B. uniformis* p3-2 was inhibited by D-xylose and D-galactose. The *bft* gene detection and the enterotoxin production were observed only in 13 EF-enterotoxigenic species. Fragilysin activity was confirmed on HT-29 cells. The antimicrobial determination confirmed that both imipenem and metronidazole were efficient against *B. fragilis* species; all the strains were resistant to lead and nickel. Plasmids of 2.9, 4.4, 4.8, and 8.9 kb were observed in 6 tested strains. These results show the values of the species identification from clinical infections, as well as of the periodic evaluation of the resistance patterns of the *B. fragilis* group at Brazilian medical institutions.

Key words: *Bacteroides fragilis* - enterotoxigenic *Bacteroides fragilis* - pathogenesis - antimicrobial resistance - plasmid

Species of the genus *Bacteroides* are important constituents of both human and animal intestinal microbiota, achieving approximately 30% of the total of organisms cultured from feces (Soki et al. 1999).

Bacteroides fragilis is considered to be the most virulent species in this genus due to its virulence factors and its predominance in monomicrobial and mixed infections such as brain, lung, intra-abdominal and intra-pelvic abscesses, peritonitis, and sepsis. Some virulence factors were noticed in *B. fragilis* such as adherence, hemagglutination, polysaccharide capsule, fimbriae, as well as antibiotic and metal ion resistances (Gorbach & Bartlett 1974, Duerden 1980).

B. fragilis also produces an enterotoxin (ETBF) that causes diarrhea in animals and it has been associated with diarrhea in human beings (Myers et al. 1984). This extracellular toxin has been characterized as a heat-labile zinc-metalloprotease of approximately 20 kDa and it resides as a pro-toxin in the periplasmic space (Moncrief et al. 1995). The *bft* chromosomal gene, which has been isolated and sequenced, encodes the toxin named fragilysin. It has been observed a toxin activity against tight junction proteins,

disrupting the intestinal epithelial barrier and causing morphologic alterations such as loss of cell-to-cell attachment, rounding, apparent swelling, and pyknosis. Moreover, this fragilysin produces the hydrolysis of gelatin, actin, azocoll, tropomiosin, and fibrinogen (Koshy et al. 1996, Franco et al. 1997, Kato et al. 2000).

In the present study, bacteria of the *B. fragilis* group isolated from stool samples of children with diarrhea were evaluated to detect putative virulence factors in vitro such as capsule, temperature and serum resistance, hemolytic activity, hemagglutination, hydrophobicity, antibiotic and metal susceptibility, and plasmid detection; the presence of the *bft* gene and the fragilysin production were also detected.

MATERIALS AND METHODS

Bacterial strains - Clinical isolates were obtained from diarrhea stool samples collected from April to December 2000 in 2 child hospitals of São Paulo, Brazil (Hospital Menino Jesus and Instituto da Criança do Hospital das Clínicas). A total of 64 strains of the *B. fragilis* group were isolated from the intestinal microbiota of 15 children with diarrhea (ranges from 2 months old to 6 years old). Bacteria were isolated and identified at the Anaerobe Laboratory, Department of Microbiology, University of São Paulo. Two positive enterotoxigenic *B. fragilis* ATCC 43858 and GAI 97124, kindly provided by Dr Annalisa Pantosti (Laboratory of Bacteriology and Medical Mycology, Institute Superiore di Sanità, Rome, Italy) and by Dr Naoki Kato (Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Gifu, Japan), respectively, were used as positive control in the enterotoxin production assay. Strains were stored in 10% skim milk at -80°C. The Ethic

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo, proc. 02/02680-4

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⁺⁺Fapesp fellowship, proc. 02/02679-6

Received 15 December 2003

Accepted 30 April 2004

Commission of the hospitals and of the Instituto de Ciências Biomédicas, USP (Parecer 158/CEP) approved this study.

Capsule detection - Capsules were detected by using a Hiss stain with modifications (Namavar et al. 1991). Each isolate was anaerobically grown in peptone-yeast extract (PY) with 1% glucose. A drop of 24-h-bacterial-suspension was deposited on a microscope slide, and it was fixed with methyl alcohol (100%), for 1 min. Then, it was covered with crystal violet and heated until vapor emission. Each slide was washed with 20% (w/v) of copper sulfate and allowed to air-dry. Capsule was identified under light microscopy (100X) through verification of a clear area around the bacteria.

Temperature resistance - Strains were grown in brain heart infusion broth (BHI, Oxoid), supplemented with yeast extract (0.5%) in anaerobiosis conditions (90% N₂/10% CO₂), at 37°C, for 48 h; aliquots of 0.1 ml were inoculated in 5 ml of fresh BHI medium and then incubated in water bath at 60°C, for 30 min, and afterwards for 1 h, and also at 100°C, for 1, 5, and 10 min (Holdeman et al. 1977). After the period of incubation, the tubes were incubated under anaerobiosis, at 37°C, for 48 h. Bacterial growth was interpreted as a heat-resistance. An inoculated BHI broth, not exposed at heat, was used as control.

Hemolytic activity - The hemolysin production was performed on trypticase soy agar (TSA, Oxoid) enriched with horse blood (5%) (Avila-Campos 1995). The strains were grown in BHI broth supplemented with yeast extract (0.5%) and incubated in anaerobiosis, at 37°C, for 48 h. Plates were inoculated in duplicate by using a Steer's replicator, with a given final inocula of ca. 10⁵ cfu/spot, and then incubated at 37°C, for 48 h. The hemolytic activity was identified through the verification of a clear zone around the bacterial growth.

Serum resistance - Serum resistance was tested using human and horse sera (Namavar et al. 1991). Each serum was pooled, and aliquots of 1 ml were stored at -20°C. Each strain grew for 24 h in BHI broth and they were washed once in 0.01 M of phosphate-buffered saline (PBS, pH 7.4); then 1 ml (1.5 x 10⁸ cfu/ml) was mixed with 1 ml of human (100%) or horse (100%) serum, and incubated in anaerobiosis, at 37°C, during 4 h. Afterwards, 0.1 ml of each mixture was removed and inoculated on blood agar with a Steer's replicator (10⁵ cfu/spot) and incubated in anaerobiosis, at 37°C, for 48 h, for viable counts. 100% of inactivated sera (56°C, 30 min) were used as control. A total bacterial inhibition was considered susceptible to serum.

Hemagglutination assays (HA) - Organisms were grown in BHI broth, in anaerobiosis, for 24 h, at 37°C, then harvested, pellet, and washed (10,000 g, 5 min) 3 times in PBS. The bacterial suspension was adjusted to ca. 1.5 x 10⁸ cfu/ml (Vel et al. 1986). Human erythrocytes, collected each week and stored at a concentration of 10% (v/v), in Alsever's solution, from a 23 year-old man, A Rh-positive donor, were harvested and then washed 3 times (600 g, 4°C, 5 min) and finally resuspended at 1% in PBS. HA was tested qualitatively by mixing 50 µl of bacterial suspension with 50 µl of erythrocyte suspension in a 96-well microtitre plate (V shaped). Two-fold serial dilutions

of the bacterial suspension of a final volume of 50 µl/well were performed. Then, 50 µl erythrocytes were added to each dilution and the plate was gently shaken, being incubated first at 37°C, for 1 h, and afterwards at 4°C, overnight. Hemagglutination activity titers were expressed through the reciprocal of the highest bacterial dilution showing agglutination activity.

Hemagglutination assay inhibition (HAI) - The ability of D-arabinose, D-galactose, D-mannose and D-xylose to inhibit hemagglutination was evaluated. Each carbohydrate was dissolved in 80 mM of PBS and then filtrated (0.45 mm, Millipore). To each 9.9 ml of each carbohydrate solution it was added 0.1 ml of washed erythrocytes. The HAI was qualitatively performed by mixing 50 µl of bacterial suspension with 50 µl of erythrocytes treated with each carbohydrate suspension. Two-fold serial dilutions of the bacterial suspension of a final volume of 50 µl/well were performed. Then, 50 µl of erythrocytes treated with carbohydrate were added and the plate was gently shaken, incubated (37°C, 1 h), and then at 4°C, overnight. Erythrocytes treated with sugar and mixed with PBS were used as negative control.

Hydrophobicity (Hp) - The hydrophobicity of the bacterial surface was measured through the interaction with hexadecane (Rosenberg et al. 1981). Strains grown in BHI broth for 24 h, at 37°C, were washed three times in phosphate-urea-magnesium buffer (PUM) and resuspended to ca. 1.5 x 10⁸ bacteria/ml. Then, 3 ml of bacterial suspension were mixed with 400 µl of n-hexadecane (Sigma Chemical Co., US) and incubated at 30°C, for 10 min. After the vortex and the separation of 2 phases, absorbance (A_{550nm}) was determined. Hp values were expressed through the percentage of bacteria recovered in the aqueous phase. Percentage values > 50% were considered as hydrophilic, and Hp < 50% as hydrophobic. These tests were performed in triplicate.

Fragilysin activity - Bacterial supernatants were used. HT-29/C₁ cells were grown in flasks of 25 cm² with 5 ml of Eagle's medium with glutamine (Gibco), supplemented with penicillin (100 UI/ml), streptomycin (100 µg/ml), and inactivated fetal bovine serum (15%); then they were incubated in air plus 5% CO₂, at 37°C. These cells were distributed (200 µl/well) into a 96-microtitration plate (Corning, US), and they were allowed to grow during 2-3 days. Before the assay, the medium was removed and 180 µl of fresh medium without serum were added to each well, and then 20 µl of supernatant were added in each well, in duplicate. The plate was incubated at 37°C, in air plus 5% CO₂ and then examined after 3-4 h, period in which the typical toxin-induced cytotoxic changes occur (Pantosti et al. 1994).

Fragilysin gene detection (bft) - From 5 to 6 colonies of each strain were suspended in 1 ml of Milli-Q water, being homogenized and boiled for 10 min, and finally centrifuged at 14,000 g, for 10 min. Supernatants were used as templates. The amplification reaction was performed by using a specific primer pair: 5' - GAC GGT GTA TGT GAT TTG TCT GAG AGA -3' and 5' - ATC CCT AAG ATT TTA TTA TCC CAA GTA -3' (Gibco BRL) (Pantosti et al. 1997). The polymerase chain reaction (PCR) was performed in a final volume of 25 µl containing 2.5 µl of 10 X PCR buffer

(Gibco), 1.25 µl of MgCl₂ (1.5 mM), 1 µl of dNTP (0.2 mM) (Gibco), 0.25 µl of *Taq* DNA polymerase (0.5 U) (Gibco), 1 µl of each primer (0.4 mM), 8 µl of Milli Q water and 10 µl of DNA. The thermocycler (Perkin Elmer Amp PCR System 2400) was programmed to: 1 cycle of 94°C (5 min), followed by 35 cycles of 94°C (1 min), 52°C (1 min), 72°C (1 min) and 1 cycle of 72°C (5 min). The amplified products were analyzed by 1% agarose gel electrophoresis, stained with 0.5 µg/ml of ethidium bromide (EtBr) and photographed by using a Kodak Digital Science System DC 120.

Antibiotic and metal susceptibility testing - An agar dilution method by using a Wilkins-Chalgren agar was used as reference (Sutter et al. 1979, Riley & Mee 1982). The antibiotics used were: amoxicillin, ampicillin, clindamycin, erythromycin, and tetracycline (Luper Ind. Farm. Ltd., SP, Brazil), amoxicillin/clavulanic acid (Smithkline Beecham Ltd., SP, Brazil), cefoxitin and imipenem (Merck, Sharp & Dohme, SP, Brazil), penicillin G (Prodoti Lab. Farm. Ltd., SP, Brazil), metronidazole (Aventis Farm. Ltd., SP, Brazil). The metals used were: mercuric chloride (HgCl₂), silver nitrate (AgNO₃), copper sulfate (CuSO₄) and nickel sulfate (NiSO₄·6H₂O) (Labsynth Prod. Lab. Ltd., SP, Brazil), cadmium sulfate (3CdSO₄·8H₂O) and lead chloride (PbCl₂) (Vetec Química Fina Ltd., SP, Brazil).

Antibiotic and metal concentrations were ranged from 0.25 to 512 µg/ml. Media without antimicrobials were used as control. Bacterial inocula were standardized to ca. 1.5 x 10⁸ cfu/ml. Each plate was inoculated by using a Steers replicator, with ca. 1.5 x 10⁵ cfu/spot. All the plates were incubated in anaerobiosis, at 37°C, for 48 h. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of each antimicrobial agent able to inhibit the macroscopic bacterial growth.

Plasmid detection - Plasmid DNA was extracted from all tested strains by using a ConcertTM Rapid Plasmid Miniprep System (Gibco BRL) from all tested strains. The DNA was analyzed by agarose gel electrophoresis. The strain *Escherichia coli* J53 pACYC 184 was used as a plasmid-positive control.

RESULTS

A total of 64 species of the *B. fragilis* group were isolated from the intestinal microbiota of 15 children with diarrhea: 26 *B. fragilis*; 13 enterotoxigenic *B. fragilis*; 8 *B. vulgatus*; 6 *B. uniformis*; 6 *B. distasonis*; and 5 *B. ovatus*.

All *B. fragilis* presented a capsule and the following characteristics: β-hemolytic, hydrophilic, and resistant to human or horse serum. Variable data were verified in the temperature resistance and in the hemagglutination assay. Twenty-four (37.5%) were resistant at 60°C, for 30 min and, among them, only 12 (18.75%) were resistant at 60°C, for 60 min. None strain was resistant at 100°C (Table I).

Four strains were able to hemagglutinate erythrocytes, showing HA titers of 8 (*B. uniformis* p3-2, *B. fragilis* p3-3) and 2 (*B. fragilis* p76a, p76b). Carbohydrates D-mannose, D-galactose, D-arabinose, and D-xylose were able to inhibit the hemagglutination of two *B. fragilis* strains (p76a, p76b). The strain *B. uniformis* p3-2 was inhibited only by D-xylose and by D-galactose. Moreover, none carbohy-

TABLE I
Temperature resistance and plasmid profile in 64 *Bacteroides fragilis* strains isolated from children's stool samples

Species	Strain nr	Resistance to 60°C		Plasmid profile	
		30 min	60 min	Band nr	kb
<i>B. fragilis</i>	4	+	+	-	-
<i>B. fragilis</i>	6	+	-	-	-
<i>B. fragilis</i>	11	-	-	-	-
<i>B. fragilis</i>	1	-	-	1	4.4
<i>B. fragilis</i>	1	-	-	2	2.9,4.4
ETBF	3	+	+	-	-
ETBF	7	-	-	-	-
ETBF	3	-	-	1	4.4
<i>B. distasonis</i>	1	+	+	-	-
<i>B. distasonis</i>	4	+	-	-	-
<i>B. distasonis</i>	1	-	-	-	-
<i>B. ovatus</i>	1	+	-	-	-
<i>B. ovatus</i>	4	-	-	-	-
<i>B. uniformis</i>	3	+	+	-	-
<i>B. uniformis</i>	1	+	-	-	-
<i>B. uniformis</i>	2	-	-	-	-
<i>B. vulgatus</i>	1	+	+	-	-
<i>B. vulgatus</i>	6	-	-	-	-
<i>B. vulgatus</i>	1	-	-	1	8.9

ETBF: *B. fragilis* - enterotoxigenic

drate was able to inhibit the HA in the strain *B. fragilis* p3-3.

The *bft* gene was detected only in 13 cytotoxic strains and the enterotoxin production was confirmed by a morphologic alteration in the HT-29/C₁ cells. All the ETBF were isolated from diarrhea stools and they produced a characteristic 294-bp band by PCR, and a cytotoxic effect, which was visible after 4 h, producing refringent-rounded cells with clear outlines and detached one from another.

The MIC values to antibiotics and metals for the 64 tested species are showed in Tables II and III, respectively. All the strains showed 100% of resistance to lead and nickel. Moreover, imipenem and metronidazole were very actives against all strains.

Plasmids of low molecular weight were observed in 3 enterotoxigenic *B. fragilis* (4.4 kb), in 1 non-enterotoxigenic *B. fragilis* (4.4 kb), in 1 non-enterotoxigenic *B. fragilis* (2.9 and 4.8 kb) and in 1 *B. vulgatus* (8.9 kb) (Table I).

DISCUSSION

In this study, all the examined *B. fragilis* strains possessed a capsule, according to Kasper et al. (1977) and Domingues et al. (1995). The capsular polysaccharide *B. fragilis* acts as a protector against neutrophil phagocytes and the complement bactericidal activity; it can produce abscesses in animals (Lindberg & Weintraub 1985). Moreover, all the tested strains showed characteristics such as β-hemolytic, hydrophilic, and human or horse serum resistant, in accordance with Rotimi and Eke (1984), Oyston and Handley (1990), and Namavar et al. (1991).

Bacterial capsules are usually hydrophilic, contributing, therefore, to the low hydrophobicity of the cellular

TABLE II
Antimicrobial activity and resistance rates of 10 antibiotics to 64 bacteria of the *Bacteroides fragilis* group

Antibiotics	Breakpoint ^a	MIC (µg/ml)			Resistance (%)
	µg/ml	Range	50%	90%	
Amoxicillin	8	4 – 256	32	256	90.5
Amoxicillin/Clavulanic acid	8	≤ 0.25 – 64	8	32	40.5
Ampicillin	4	≤ 0.25 – 256	16	256	92
Cefoxitin	16	≤ 0.25 – 256	16	32	23.5
Clindamycin	8	≤ 0.25 – 256	≤ 0.25	256	34.5
Erythromycin	8	≤ 0.25 – 256	256	256	97
Imipenem	16	≤ 0.25	≤ 0.25	≤ 0.25	0
Metronidazole	16	≤ 0.25 – 4	≤ 0.25	2	0
Penicillin G	8	1 – 256	32	256	87.5
Tetracycline	8	≤ 0.25 – 32	16	16	51.5

a: breakpoints used from NCCLS (1997)

TABLE III
Antimicrobial activity and resistance rates of six metals to 64 bacteria of the *Bacteroides fragilis* group

Metals ^a	MIC (µg/ml)			Resistance (%)
	Range	50%	90%	
HgCl ₂	≤ 0.25 – 8	4	8	59
AgNO ₃	≤ 0.25 – 32	16	32	70
CuSO ₄	≤ 0.25 – 256	16	64	68.5
3CdSO ₄ ·8H ₂ O	≤ 0.25 – 128	16	128	68.5
PbCl ₂	64 – ≤ 512	512	≤ 512	100
NiSO ₄ ·6H ₂ O	128 – ≤ 512	512	≤ 512	100

a: the breakpoint for all metals was 2 mg/ml (Avila-Campos et al. 1991). Susceptibility for *B. fragilis* ATCC 43858: HgCl₂, 4 µg/ml; AgNO₃, 8 µg/ml; CuSO₄, 32 µg/ml; 3CdSO₄·8H₂O, 16 µg/ml; PbCl₂, ≤ 512 µg/ml and NiSO₄·6H₂O, ≤ 512 µg/ml. Susceptibility for *B. fragilis* GAI 97124: HgCl₂, 4 µg/ml; AgNO₃, 0.5 µg/ml; CuSO₄, 8 µg/ml; 3CdSO₄·8H₂O, ≤ 0.25 µg/ml; PbCl₂, ≤ 512 µg/ml and NiSO₄·6H₂O, 256 µg/ml

surface (Oyston & Handley 1990). In our study, it was not observed any correlation between the presence of capsule and hydrophobicity.

The serum bactericidal activity is perhaps the most important host's resistance factor against the systemic bacterial invasion. Thus, the resistance of the *B. fragilis* strain against human or horse sera may explain its pathogenic potential in severe clinical infections (Rotimi & Eke 1984). Namavar et al. (1991) showed that avirulent *B. fragilis* strains were more sensitive to serum than the virulent ones, indicating that the complement is crucial for killing of avirulent strains.

Therefore, the resistance at 60°C, for 60 min, observed in 12 tested strains, suggests an association with the presence of capsule. Four strains (*B. uniformis* p3-2; *B. fragilis* p3-3; *B. fragilis* p76a; *B. fragilis* p76b) produced hemagglutination. Oyston and Handley (1990) detected different hemagglutination patterns among *B. fragilis* strains, and these differences were not observed in this study. There was not any correlation between capsulation (as

seen with Hiss stain) and hemagglutination in accordance with Namavar et al. (1991). Moreover, D-mannose, D-galactose, D-arabinose or D-xylose was able to inhibit HA in these organisms.

Domingues et al. (1991) analyzed 31 *B. fragilis* isolated from intestinal microbiota and other infections, and they observed that 84.2% of clinical strains were HA-positive. On the other hand, Oyston and Handley (1990) observed that one *B. fragilis* strain produced human or horse erythrocytes agglutination, and 13 strains hemagglutinated rabbit's erythrocytes. These conflictive data about HA in *B. fragilis* have been observed, however, it can be suggested that adhesins or outer membrane proteins could be responsible for hemagglutination (Vel et al. 1986, Oyston & Handley 1990).

By PCR, 13 ETBF showed a 294-bp band and they also produced morphologic alterations in the HT-29/C₁ cells. PCR assay proved to be a good methodological choice in the detection of fragilysin, due to its speed and ease of reading. The evaluation of the enterotoxin activity by the HT-29/C₁ cells assay was successful. Studies have shown that ETBF were recovered in 3.1% from feces of immunodeficient children (Bressane et al. 2001) and in 2.1% in children with diarrhea (Krzyzanowsky & Avila-Campos 2003). Moreover, Cáceres et al. (2000) showed the presence of ETBF in 8.4% of Nicaraguan children.

This fragilysin produced rounded and refringent cells with clear outlines and they were detached one from another. In this study, all the 13 ETBF strains isolated from diarrhea stools produced a characteristic rounded of the HT29/C₁ cells. Moreover, studies in vivo have showed that ETBF produce an accumulation of sodium, chloride, albumen, and protein. Despite of this fact, ETBF strains have been considered as enteropathogens to children and adults in several countries (Pantosti et al. 1994, 1997). However, it seems to be too early to suggest that this protease is a determinant factor of the *B. fragilis* invasiveness, and more studies are needed, according to Ferreira et al. (1999).

The bacteria of the *B. fragilis* group have been studied due to their pathogenic character and their resistance

to several antibiotics and heavy metals. All isolates were resistant to lead and nickel. Studies have documented an alarming and gradual increase of the resistance to several antimicrobials in anaerobes, particularly in *Bacteroides* species (Turner et al. 1995, Snyderman et al. 1996). In Brazil, Avila-Campos et al. (1991), Carvalho et al. (1997), and Pestana et al. (1999) have analyzed the antimicrobial susceptibility of *B. fragilis* isolated from both human and *Callithrix penicillata* marmoset gastrointestinal tracts showing an increase of resistance to drugs.

Bacteria of the *B. fragilis* group have shown an interesting multiple resistance to metronidazole, imipenem, and amoxicillin/clavulanic acid (Turner et al. 1995). On the other hand, *Bacteroides* strains are β -lactamases producers, which are able to hydrolyze most of the β -lactam drugs.

Data regarding metal susceptibility in *B. fragilis* group bacteria are few. However, metal ions resistance in organisms has gradually increased due to the use of metals in industry, agriculture, hospitals, and medicines. Moreover, Riley and Mee (1982) and Avila-Campos et al. (1991) have verified the metal ion resistance in *B. fragilis* isolated from human infections.

None plasmid-positive strains (*B. fragilis* p15j with plasmid of 4.4 kb, *B. vulgatus* p23j with 8.9 kb, *B. fragilis* p35a with 4.8 and 2.9 kb, *B. fragilis* p35b with 4.4 kb, *B. fragilis* p60c with 4.4 kb and *B. fragilis* p60h with 4.4 kb) lost these elements after the EtBr treatment. Pestana et al. (1999) showed that from 5 *B. fragilis* strains, only 1 strain harbored plasmid, which was associated to the resistance to penicillin and clindamycin. However, several studies have shown that *Bacteroides* spp. contain transferable plasmids (Tally et al. 1979, Welch et al. 1981). Whether or not, the connection between the resistance to some heavy metals and the presence of plasmid in *Bacteroides* spp. remains to be investigated.

Plasmids of low molecular weight are often observed in many *Bacteroides* spp. recovered from infections or from the indigenous fecal microbiota (Beul et al. 1985). Wallace et al. (1981) showed that 25% of clinical *Bacteroides* isolates contained three different plasmids (5, 3, and 2 MDa), and Soki et al. (1999) showed 5.5 and 4.2 kb plasmids among *Bacteroides* spp. isolated from both clinical isolates and fecal samples of healthy donors.

According to Wallace et al. (1981) the resistance to metal ions in *Bacteroides* spp. is intrinsic rather than plasmid borne. In this study, we observed plasmids with low weight. The occurrence of small cryptic plasmids with the same molecular size in different *Bacteroides* strains in different parts of the world may be due to their horizontal mobilization by several different conjugative elements which may promote their spread through the indigenous microbiota (Smith & Parker 1996).

Species of the *B. fragilis* group are important organisms in the pathogenesis of clinical infections and a periodic evaluation of the virulence factors and antimicrobial susceptibility are suggested. Moreover, the wide distribution of plasmids may signal a potential problem if they acquire an antibiotic resistance or pathogenicity determinants, which may render the treatment of bacteroides infections more difficult.

ACKNOWLEDGMENTS

To Ms Zulmira Alves de Souza for her technical support.

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