Enterotoxigenic and nontoxigenic *Bacteroides fragilis* strains isolated in Brazil

Karla R Miranda/+, Mariana F Dias, Priscilla LS Guimarães, Renata F Boente, Heidi Pauer, Priscila Z Ramos, Laís S Falcão, Eliane de O Ferreira, Ilana T Balassiano/[1], Livia Q Ferreira, Joaquim dos Santos-Filho, Geraldo R de Paula[2], Eduardo NF Antunes[1], Katia ES Avelar[1], Regina MCP Domingues

Laboratório de Biologia de Anaeróbios, Departamento de Microbiologia Médica, Instituto de Microbiologia Prof. Paulo de Góes, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 3737, 21941-900 Rio de Janeiro, RJ, Brasil [1]Laboratório de Zoonoses Bacterianas, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil [2]Departamento de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade Federal Fluminense, Niterói, RJ, Brasil

The presence of enterotoxigenic Bacteroides fragilis *and nontoxigenic* B. fragilis *(NTBF) among 109 strains isolated from 1980-2008 in Brazil were investigated by PCR. One strain, representing 0.9% of the total analyzed strains, harbored the* bft *gene which was identified as* bft*-1 isoform based on PCR-RFLP and sequencing. Forty-nine strains (44.9%) exhibited the NTBF pattern III which possesses the flanking region required for pathogenicity island acquisition in which the* bft *gene is codified. These data reinforce the potential of* B. fragilis *as an emerging enteropathogen in our country.*

Key words: *Bacteroides fragilis* toxin - enterotoxigenic *Bacteroides fragilis* - nontoxigenic *Bacteroides fragilis*

Bacteroides fragilis is a Gram-negative obligate anaerobic bacterium and constitutes about 1% of the normal faecal flora of humans (Drasar & Duerden 1991). The bacterium is usually isolated from endogenous infections (Eribe & Olsen 2000) and its pathogenicity has been attributed to several virulence determinants, including an enterotoxin encoded by the *bft* gene (Sears et al. 2006). According to the presence of a *B. fragilis* Pathogenicity Island (BfPAI) and its flanking region, *B. fragilis* strains can be classified mainly in: (1) enterotoxigenic *B. fragilis* (ETBF) strains, or pattern I, containing BfPAI and its flanking region within the conjugative transposon CTn86 (~65 kb); (2) nontoxigenic *B. fragilis* (NTBF) strains, or pattern II, lacking the BfPAI as well as a flanking CTn; and (3) NTBF strains, or pattern III, that lack the BfPAI but contain conjugative transposons, either CTn9343 or variants of CTn9343 or CTn86 (Moncrief et al. 1995, Franco et al. 1999, Franco 2004, Buckwold et al. 2007). The detection of the *bft* gene in a BfPAI embedded in a CTn suggests that the toxin gene can be passed by horizontal gene transfer events from ETBF strains to NTBF strains of pattern III (Moncrief et al. 1995).

In order to follow the evolution of enterovirulence profiles in Brazil, we determined in the present study the distribution of ETBF and NTBF patterns in 109 *B. fragilis* strains obtained between 1980-2008 from several hospitals in Rio de Janeiro. All strains were cultivated anaerobically following recommendations of Jousimies-Sommer et al. (2002). *B. fragilis* ATCC 43859 and ATCC 23745 strains, respectively, were used as positive and negative controls for *bft* gene detection by PCR.

Extraction and purification of DNA were performed according to Pitcher et al. (1989). Amplification conditions were the same as reported by Scotto d'Abusco et al. (2000). Primers BF-5 (5' -GATGCT CCAGTTACAGCT-TCCATTG-3') and BF-6 (5' -CGCCCAGTATATGAC-CTAGTTCGTG-3') were used to amplify a 976-bp internal fragment of the three isoforms of the *bft* gene (pattern I) (Scotto d'Abusco et al. 2000). A fragment of expected size was amplified from one *B. fragilis* strain (0.9%) which was classified as ETBF. Other studies in Brazil already reported low numbers of ETBF strains, ranging from 1.5-3.0% (Bressane et al. 2001, Antunes et al. 2002, Krzyzanowsky & Avila-Campos 2003). Nevertheless, high numbers of ETBF strains have been isolated in several other regions of the world (Kato et al. 1996, Pantosti et al. 1997). For instance, ETBF represented 18.6% of the strains isolated in Japan (Kato et al. 1996) while in Poland and in Netherlands rates of approximately 14% were found (Obuch-Woszczatyński et al. 2004).

For amplification reactions of the 12-kb regions flanking BfPAI, we used primers P1T3 (5' - TTCAAC-CTGATCGATCCGGAAGATCCG- 3') and P1T7 (5' - GGTAGACTACCTGAGTAAGGAGTC-3'), that yield a 1.6 kb fragment in NTBF pattern III strains (Scotto d'Abusco et al. 2000). A 1.6kb fragment was amplified from 49 strains (44.9%). NTBF III was the most frequent pattern and was present in 40.8-55.5% of the samples (Table).

By using PCR restriction fragment length polymorphism to classify *bft* isoforms (Scotto d'Abusco et al. 2000), the ETBF strain isolated was shown to harbor the *bft*-1 isoform (data not shown). Moreover, the amplified

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+ Corresponding author: karlarodr@yahoo.com.br
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TABLE

Distribution of *Bacteroides fragilis* patterns between the 109 strains isolated in Brazil from 1980-2008

	1980-1989		1990-1999		2000-2008	
	n	%	n	%	n	%
ETBF	0	0	0	0	1	2.3
NTBF III	10	55.5	20	40.8	19	45.2
Total[a]	18	100	49	100	42	100

a: total of strains analyzed per period; ETBF: enterotoxigenic *Bacteroides fragilis*; n: number of positive strains; NTBF: nontoxigenic *Bacteroides fragilis*.

bft gene fragment was purified (Illustra GFX™ PCR DNA and Gel Band Purification Kit, GE Healthcare) and sequenced at the Human Genome Research Center (Institute of Biosciences, University of São Paulo) by using the MegaBACE™1000 and DYEnamic ET Dye Terminator Cycle Sequencing Kit (Thermo Sequenase™ II DNA Polymerase). The sequence was edited with the BioEdit Sequence Alignment Editor version 7.0.1 and analyzed by Blast software (www.ncbi.nlm.nih.gov/blast) against sequences deposited in GenBank. The nucleotide sequence of the *bft* gene showed 99.9% similarity with the deposited *bft*-1 sequence. This result is in agreement with several similar studies where the *bft*-1 allele was found to be the most predominant isotype (Moncrief et al. 1995, Ulger et al. 2006, Avila-Campos et al. 2007).

In summary, only one single ETBF strain was identified among 109 Brazilian samples used in this study. This result is in agreement with several other reports that demonstrated a low incidence of ETBF in our country. Based on the percentage of NTBF III detected and assuming a possible horizontal transfer, further investigations are required to monitor eventual changes in the enterovirulence pattern of this microorganism.

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REFERENCES

Antunes ENF, Ferreira EO, Vallim DC, Paula GR, Seldin L, Sabrá A, Ferreira MCS, Domingues RMCP 2002. Pattern III non-toxigenic *Bacteroides fragilis* (NTBF) strains in Brazil. *Anaerobe 8*: 17-22.

Avila-Campos MJ, Liu C, Song Y, Rowlinson M-C, Finegold SM 2007. Determination of *bft* gene subtypes in *Bacteroides fragilis* clinical isolates. *J Clin Microbiol 45*: 1336-1338.

Bressane MA, Durigon LE, Avila-Campos MJ 2001. Prevalence of the *Bacteroides fragilis* group and enterotoxigenic *Bacteroides fragilis* in immunodeficient children. *Pathogenesis 7*: 277-281.

Buckwold SL, Shoemaker NB, Sears CL, Franco A 2007. Identification and characterization of conjugative transposes CTn86 and CTn9343 in *Bacteroides fragilis* strains. *Appl Environ Microbiol 73*: 53-63.

Drasar BS, Duerden BI 1991. *Anaerobes in the normal flora of man*. In BI Duerden, BS Drasar, *Anaerobes in Human Disease*, Wiley-Liss, New York, p. 162-179.

Eribe ER, Olsen I 2000. Strain differentiation in *Bacteroides fragilis* by ribotyping and computer-assisted gel analysis. *APMIS 108*: 429-438.

Franco AA 2004. The *Bacteroides fragilis* pathogenicity island is contained in a putative novel conjugative transposon. *J Bacteriol 186*: 6077-6092.

Franco AA, Cheng RK, Chung G-T, Wu S, Oh H-B, Sears CL 1999. Molecular evolution of the pathogenicity island of enterotoxigenic *Bacteroides fragilis* strains. *J Bacteriol 183*: 6623-6633.

Jousimies-Sommer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM 2002. *Wadsworth-KTL Anaerobic Bacteriology Manual*, 6th ed., Star Publishing, Belmont, 287 pp.

Kato N, Kato H, Watanabe K, Ueno K 1996. Association of enterotoxigenic *Bacteroides fragilis* with bacteremia. *Clin Infect Dis 23* (Suppl. I): 83-86.

Krzyzanowsky F, Avila-Campos MJ 2003. Detection of non-enterotoxigenic and enterotoxigenic *Bacteroides fragilis* in stool samples from children in São Paulo, Brazil. *Rev Inst Med Trop São Paulo 45*: 225-227.

Moncrief JS, Obiso R, Barroso LA, Kling JJ, Wright RL, Van Tassell RL, Lyerly DM, Wilkins TD 1995. The enterotoxin of *Bacteroides fragilis* is a metalloprotease. *Infect Immun 63*: 175-181.

Obuch-Woszczatyński P, Wintermans RG, Van Belkum A, Endtz H, Pituch H, Kreft D, Meisel-Mikołajczyk F, Luczak M 2004. Enterotoxigenic *Bacteroides fragilis* (ETBF) strains isolated in The Netherlands and Poland are genetically diverse. *Pol J Microbiol 53*: 35-39.

Pantosti A, Menozzi MG, Frate A, Sanfilippo L, D'Ambrosio F, Malpeli M 1997. Detection of enterotoxigenic *Bacteroides fragilis* and its toxin in stool samples from adults and children in Italy. *Clin Infect Dis 24*: 12-16.

Pitcher DG, Saunders NA, Owen RJ 1989. Rapid extraction of bacterial genomic DNA with guanidine thiocyanate. *Lett Appl Microbiol 8*: 151-156.

Scotto d'Abusco A, Grosso MD, Censini S, Covacci A, Pantosti A 2000. The alleles of the *bft* gene are distributed differently among Enterotoxigenic *Bacteroides fragilis* strains from human sources and can be present in double copies. *J Clin Microbiol 38*: 607-610.

Sears CL, Buckwold SL, Shin JW, Franco AA 2006. The C-terminal region of *Bacteroides fragilis* toxin is essential to its biological activity. *Infect Immun 74*: 5595-5601.

Ulger Toprac N, Rajendram D, Yagci A, Gharbia S, Shah HN, Gulluoglu BM, Akin LM, Demirkalem P, Celenk T, Soyletir G 2006. The distribution of the *bft* alleles among enterotoxigenic *Bacteroides fragilis* strains from stool specimens and extraintestinal sites. *Anaerobe 12*: 71-74.