Enterotoxinogenic and nontoxigenic Bacteroides fragilis strains isolated in Brazil

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The presence of enterotoxinogenic 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 - enterotoxinogenic 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-Somer 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 CCAGTACGCTTCCATGG-3’) and BF-6 (5’-GGCCCAGTATATGACCTAGTTCGTG-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 PIT3 (5’-TTCAACTCTGATCGATCCCGAAGATCCGG-3’) and PIT7 (5’-GTTAGACTACCTGAGTAAGGAGTC-3’), that yield a 1.6 kb fragment in NTBF pattern III strains (Scotto d’Abusco et al. 2000). A 1.6 kb 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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TABLE

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

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<tbody>
<tr>
<td>ETBF</td>
<td>n %</td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>NTBF III</td>
<td>0 0</td>
<td>0 0</td>
<td>1 2.3</td>
</tr>
<tr>
<td>Total</td>
<td>18 100</td>
<td>49 100</td>
<td>42 100</td>
</tr>
</tbody>
</table>

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

The 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 with several similar studies where the bft-1 sequence. This result is in agreement with several 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 its toxin in stool samples from children in São Paulo, Brazil. Rev Inst Med Trop São Paulo 45: 225-227.

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REFERENCES

