Coincidence of bft and cfiA genes in a multi-resistant clinical isolate of Bacteroides fragilis

Although a common member of the normal human gut flora, Bacteroides fragilis possesses various virulence factors, such as capsule, LPS and special surface proteins, and certain types of strains may produce an extracellular enterotoxin, termed fragilysin (BFT). The fragilysin-encoding gene (bft) has three different isoforms (termed bft-1, bft-2 and bft-3) (Chung et al., 1999; Franco et al., 1997; Kling et al., 1997). Because of the increasing number of resistant B. fragilis isolates, the therapeutic possibilities have been restricted to only some antibiotics, such as imipenem, tobramycin and cefoxitin (Podglajen et al., 1994; Rasmussen et al., 1987) were tested for use in PCR. During these amplification reactions, standard PCR reaction mixture was used, as described previously by Sokí et al. (2006). Of the examined IS elements, only IS614 was detected by PCR. Partial nucleotide sequence data of the upstream region of the cfiA gene by Up2, G, IS614-1I and IS614-2I primers, as described by Sokí et al. (2004), confirmed that it is an IS614B element.

B. fragilis strain R19811 contained plasmids with an estimated molecular mass of 5.6 and 9.9 kb using HindIII restriction enzyme (Pharmacia Biotech) analysis. In spite of the fact that cfiA gene could be on a plasmid (Bandoh et al., 1992; Nakano et al., 2004), in our case, Southern blot hybridization, showed it was not.

In spite of the observed metronidazole resistance, nim genes commonly responsible for metronidazole resistance in the B. fragilis group could not be detected using the nim-3 and nim-5 primer pair (Trinh & Reysset, 1996), which is suitable for the detection for all described nim genes. The annealing temperature of nim PCR was reduced from 62 to 52 °C to show incidental sequence variation in the nim gene, but PCR products could not be detected. Further investigations are necessary to establish the metronidazole resistance mechanism in this organism.

In numerous earlier studies, the presence of the cfiA gene was never observed together with the cepA gene encoding endogenous class A β-lactamase, because cfiA-positive and cepA-positive strains belong to two genetically distinct groups. The first group was characterized by the presence of cfiA gene, while in the second group, the presence of the cepA gene is typical with or without the presence of the bft gene. In spite of this observation, in 2005, Ayala et al. (2005) described two B. fragilis strains (7160 and 213E), which

we found a multi-resistant B. fragilis strain R19811 isolated from a blood culture and identified by rDNA RFLPs in the Anaerobe Reference Laboratory, Cardiff (Wareham et al., 2005). This strain co-harboured the cfiA and bft genes; therefore, our aim was to characterize its genetic background. B. fragilis strain R19811 and control strains were cultured as previously described (Sóki et al., 2000). The list and the description of the control strains have been described before (Sóki et al., 2006).

MICs to a range of antibiotics were determined by using the E-test method (AB Biodisk) according to the manufacturer’s instructions. B. fragilis strain R19811 was multi-resistant to penicillin G (MIC ≥ 256 µg ml⁻¹), amoxicillin/clavulanic acid (MIC ≥ 256 µg ml⁻¹), cefoxitin (MIC ≥ 256 µg ml⁻¹), imipenem (MIC ≥ 256 µg ml⁻¹), clindamycin (MIC ≥ 256 µg ml⁻¹) and metronidazole (MIC=64 µg ml⁻¹).

Because of the imipenem resistance, the presence of cfiA gene encoding metallo-β-lactamase was tested. cfiA PCR (Podglajen et al., 1992; Thompson & Malamy, 1990) revealed that the strain carried the cfiA gene, at the same time the presence of bft gene was also detected by PCR according to Pantosti et al. (1997).

We analysed the upstream region of the cfiA gene to detect IS elements, hypothesizing that the occurrence of one of these sequences may contribute to the development of carbenapen resistance (Podglajen et al., 1992).

By using various molecular typing methods, such as arbitrarily primed-PCR, PFGE and multilocus enzyme electrophoresis, bft-positive, cfiA-negative strains and bft-negative, cfiA-positive strains belonged to two different DNA homology groups, and the coexistence of bft and cfiA genes was not detected (Gutacker et al., 2000; Vallim et al., 2002).

During examination of the prevalence of bft- or cfiA-positive B. fragilis strains isolated from various clinical specimens,
possess both the cepA and the cfIA gene. In strain R19811, no amplification product was detected with cepA PCR as described by Gutacker et al. (2000). This suggests that the strain originally belonged to the cfIA-positive group.

In the cytotoxicity assay, as described by Pantosti et al. (1994), B. fragilis strain R19811 caused a reversible cytotoxic effect on the HT-29 cell line, this result indicated that in the cell culture supernatant, functional toxin, namely fragilysin was present. PCR-RFLP analysis of the bft gene by BTT1 (5'-CATGTCTAATGAAGCTGATTC-3') and BTT2 (5'-ATCGCCATCTGCTGTTTCCC-3') primers, with minor modification according to Chung et al. (1999), revealed a bft-1 allele.

To clarify the epidemiological background of this strain, enterobacterial repetitive intergenic consensus (ERIC) PCR typing (Versalovic et al., 1991) was applied in the case of bft-positive and cfIA-negative, bft-negative and cfIA-positive, bft- and cfIA-negative strains, and the PCR patterns were compared with the result of ERIC PCR in B. fragilis strain R19811. Two homology groups of B. fragilis can be distinguished by using molecular typing methods as described previously by Gutacker et al. (2000), Moraes et al. (1999) and Ruimy et al. (1996): the first group is characterized by the presence of the cfIA gene, these strains were bft-negative, while the strains in the second group carried only the bft gene (Fig. 1). ERIC PCR typing suggested that strain R19811, which harboured the bft and cfIA gene simultaneously, may be related to the first group, which contained only cfIA-positive strains (Fig. 1).

In summary, analysis of the results of cfIA and cepA PCR, and ERIC typing pattern, indicated that B. fragilis strain R19811 originally carried the cfIA gene and acquired the bft gene only later. Franco (2004) described two new related conjugal transposons (CTn86 and CTn9343) in connection with the bft gene. Recently, a more divergent picture has arisen. The two conjugal transposons differ in two regions: one, BPAI, found exclusively on CTn86 and harbouring the bft genes, and the other exclusively harbouring an additional 7 kb region and found on CTn9343. However, these two additional regions with the left-end of the transposons may result in hybrid elements. Still, the presence of CTn86 is mainly characteristic for bft genes and the pattern I of B. fragilis strains, while CTn9343 and the CTn9343-like elements mainly result in pattern III. The cfIA gene, however, was found only in pattern II and some pattern III isolates, but still in division II (Buckwold et al., 2007). If genes responsible for the transfer of these CTns are functional, it is possible that the bft gene can be transferred from an enterotoxigenic strain to a nontoxigenic strain. The presence of new transmissible CTns, and virulence and resistance genes, may contribute to the enhancement of the pathogenicity potential and the fitness of this strain; therefore, the aforementioned factors may promote the development of more severe infection, which may be unresponsive to the majority of the therapeutic possibilities.

**Acknowledgements**

This work was supported by grants KMA0304 and T037475 from Ministry of Economy and Transport, Hungary and the Hungarian National Research Foundation, respectively.

**Correspondence:** Elisabeth Nagy (nagyel@mlab.szote.u-szeged.hu)

---

**Fig. 1.** Comparative ERIC1-2 PCR pattern of B. fragilis strain R19811. Two groups of B. fragilis strains with similar patterns are indicated: the cfIA- and bft-positive strain R19811 in triplicate (first group) and bft-negative, cfIA-positive strains (second group). Lanes 1–3, cfIA- and bft-positive B. fragilis strain R19811; lanes 4–6, bft-positive and cfIA-negative isolates; lanes 7–9, bft-negative strains and cfIA-positive strains; lanes 10–11, bft-negative and cfIA-negative B. fragilis strains; lane 12, Bacteroides thetaiotaomicron; lane 13, ‘Bacteroides variabilis’. M, 100 bp ladder (Sigma; 100–1000 bp).


