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Bacteriology

New *cfiA* variant and novel insertion sequence elements in carbapenem-resistant *Bacteroides fragilis* isolates from Korea☆

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Abstracts

Of 276 nonduplicate *Bacteroides fragilis* clinical isolates recovered from 1997 to 2004, 3 were resistant to carbapenem. *cepA* and *cfiA* alleles were detected by polymerase chain reaction in 240 (87.0%) and 11 (4.0%) of the isolates, respectively. Insertion sequence (IS) elements were found only in the 3 carbapenem-resistant *B. fragilis* isolates, which produced metallo-β-lactamase at a level detectable by UV spectrophotometry. Sequence analysis showed 1 new *cfiA* variant, $cfiA_{11}$, and 2 novel IS elements. The $cfiA_{11}$ gene revealed 5 amino acid substitutions compared to *cfiA*, with 97.6% amino acid identity. The transposase, terminal inverted repeat sequence, and target site duplication sequence of the 2 novel IS elements were unique. This study reconfirmed the correlation between ISs and carbapenem resistance in *B. fragilis*.
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Keywords: *Bacteroides fragilis*; $cfiA_{11}$; Novel IS elements; Metallo-β-lactamase

1. Introduction

Members of the *Bacteroides fragilis* group are the most commonly encountered anaerobes in clinical specimens and are more virulent and resistant to antimicrobial agents than most other anaerobes. *B. fragilis* is of the greatest clinical significance because it produces β-lactamase or enterotoxin (Citron et al., 2007).

Three β-lactamase genes have been identified in *B. fragilis*: *cepA*, *cfxA*, and *cfiA*. Most *B. fragilis* isolates have the *cepA* gene, encoding a class A cephalosporinase, which confers resistance to various β-lactams with the exception of cephamycins and carbapenems (Rogers et al., 1994). Recently, *cfxA*, another class A cephalosporinase, was reported to be the main determinant for β-lactamase expression associated with IS*614B* in *Bacteroides* strains (Garcia et al., 2008). *cfiA* is a chromosomal class B metallo-β-lactamase gene from *B. fragilis* (Podglajen et al., 1995) and, when insertion sequence (IS) elements are present, can reduce susceptibility to carbapenems (Bandoh et al., 1991; Khushi et al., 1996; Walsh et al., 2005). Recent studies demonstrated that IS elements, such as IS*1186*, IS*942*, IS*4351*, IS*1187*, IS*1188*, IS*1169*, IS*613*, and IS*614B*, promote the expression of *cfiA*, which is located immediately downstream (Kato et al., 2003; Podglajen et al., 2001). However, as far as we know, the carbapenem resistance mechanisms of Korean *B. fragilis* isolates have not been studied.

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Table 1
Primers used for detection and sequencing of the *cepA*, *cfxA,* and *cfiA* genes and IS element

Primer	Sequence	Usage	Reference
cepA-1	5′-TTT CTG CTA TGT CCT GCC C-3′	*cepA* detection	Gutacker et al. (2000)
cepA-2	5′-ATC TTT CAC GAA GAC GGC-3′	*cepA* detection	Gutacker et al. (2000)
cfxA1y	5′-TGT GAG CTG CAA GAA CAC CA-3′	*cfxA* detection, sequencing	This study
cfxA2y	5′-CAT CTT GGT ATT TTC ATT GTT CCA-3′	*cfxA* detection, sequencing	This study
EdwF	5′-TCC ATG CTT TTC CCT GTC GCA GTT AT-3′	*cfiA* detection, sequencing	Khushi et al. (1996)
EdwR	5′-GGG CTA TGG CTT TGA AGT GC-3′	*cfiA* detection, sequencing	Khushi et al. (1996)
GBI-3	5′-CGA ACC AGA TGA CGA TAG AC-3′	*cfiA* sequencing	Kato et al. (2003)
GBI-6	5′-AAA GCA TCC GGC AAT CGT TA-3′	*cfiA* sequencing	Kato et al. (2003)
G	5′-CGC CAA GCT TTG CCT GCC ATT AT-3′	IS element detection, sequencing, *cfiA* sequencing	Podglajen et al. (1994)
E	5′-CTT CGA ATT CGG CGA GGG ATA CAT AA-3′	IS element detection, sequencing, *cfiA* sequencing	Podglajen et al. (1994)
IS1 intF	5′-TTC GTA CTC TAT CCC GTT A-3′	IS element sequencing	This study
IS1 intR	5′-CCA TCT GAT GAA ACA CCT CT-3′	IS element sequencing	This study
IS2 intF	5′-TTC GTT TCT GTC TTT GAA TC-3′	IS element sequencing	This study
IS2 intR	5′-GGC TGA CAC ACT CAA TAC CT-3′	IS element sequencing	This study
cfiA F	5′-AGT CAC CAC GTT TAT CCC GAA C-3′	Quantitative real-time PCR	This study
cfiA R	5′-ACA CCT TTC YTT TGC AGG TAA CC-3′	Quantitative real-time PCR	This study
cfiA-probe	5′-CCG CCA ATA CAA TCG CCG TGC CAG T-3′	Quantitative real-time PCR	This study

In this study, we report 3 carbapenem-resistant *B. fragilis* isolates from Korea and determine the level of *cfiA* expression using quantitative real-time polymerase chain reaction (PCR). The aims of the study were to characterize the mechanisms of carbapenem resistance and β-lactamase in *B. fragilis* isolates. This study was presented in part at the sixth conference of the Korean Society of Clinical Microbiology, Seoul, in 2003.

2. Materials and methods

2.1. Antimicrobial susceptibility testing

B. fragilis strains were collected from various clinical specimens in a Korean university hospital from 1997 to 2004. We tested specimens for imipenem susceptibility using the agar dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2004).

2.2. Detection and sequencing of cepA, cfxA, and cfiA in B. fragilis

cepA, *cfxA*, and *cfiA* were detected by PCR using the primers listed in Table 1. DNA was extracted by boiling the bacterial cell suspensions for 10 min. Reaction conditions were as follows: 35 cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min for *cepA* (Gutacker et al., 2000); 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min for *cfxA*; and 35 cycles of 92 °C for 1 min, 50 °C for 2 min, and 72 °C for 2 min for *cfiA* (Khushi et al., 1996).

To analyze the *cfiA* sequence, we obtained amplified products using 3 pairs of primers: pairs of EdwF and EdwR, and GBI-3 and GBI-6 for detection of *cfiA*, pair of G and E primers for detection of IS elements; 1.6-kb PCR product was found in isolates with IS element; otherwise, 400-bp product was found (Kato et al., 2003; Khushi et al., 1996; Podglajen et al., 1994). DNA sequencing was performed using primers in Table 1 by the dideoxy chain-termination method with an ABI 3700 Autosequencer using the reagents supplied by its manufacturer (Perkin-Elmer Cetus, Foster City, CA). The nucleotide sequences and deduced amino acid sequences were analyzed using ClustalX version 1.8 (http://www.clustal.org/download/1.X/ftp-igbmc.u-strasbg.fr/pub/ClustalX/). We compared the sequences we obtained to reference sequences using BLAST (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi). Putative promoter sequences were found according to the previous studies (Bayley et al., 2000; Kato et al., 2003; Walsh et al., 2005). The nucleotide sequences of YMC 00/5/B3685 and YMC 00/6/P496 have been deposited under the accession numbers AY372695 and AY372696, respectively.

2.3. β-Lactam hydrolytic activity of cfiA-positive isolates

The carbapenemase activities of cell sonicates from overnight Luria–Bertani broth cultures were determined with a spectrophotometer (UV-1601PC; Shimadzu, Tokyo, Japan) using either 100 μmol/L solutions of imipenem (Choong Wae, Seoul, Korea) or meropenem (Sumitomo, Tokyo, Japan) in 50 mmol/L phosphate buffer (pH 7.0) at 30 °C. The wavelengths used were 297 nm for both imipenem and meropenem. One unit of enzyme activity was defined as the amount of enzyme needed to hydrolyze 1 μmol of substrate per min at 30 °C. The protein content of the sonicates was determined with a commercial assay reagent (Bio-Rad, Hercules, CA). Inhibition method was performed as previous study; briefly, 1 mmol/L of EDTA was mixed with the enzyme for 5 min at 22 °C (Cuchural et al., 1986).

2.4. Reverse transcriptase-PCR experiments

Total RNA was extracted from the isolates of *B. fragilis* using RNeasy Protect Bacteria Mini Kit and RNase-free DNase set (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcriptase (RT)-PCR

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