Differentiation of division I (*cfiA*-negative) and division II (*cfiA*-positive) *Bacteroides fragilis* strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is increasingly used in clinical microbiological laboratories to identify bacteria and fungi at a species level and to subtype them. The *cfiA* gene encoding the unique carbapenemases found in *Bacteroides* is restricted to division II *Bacteroides fragilis* strains. The aim of this study was to evaluate whether MALDI-TOF MS is suitable for differentiating *B. fragilis* strains which harbour the *cfiA* gene from those that do not. A well-defined collection of 40 *B. fragilis* isolates with known imipenem MICs (0.062–>32 mg l⁻¹) were selected for this study. Twelve *B. fragilis* strains with known *cfiA* status, including NCTC 9343 (division I) and TAL3636 (division II), were measured by means of microflex LT MALDI-TOF MS and well-defined differences in mass spectra between the *cfiA*-positive and *cfiA*-negative strains were found in the interval 4000–5500 Da. A further 28 strains were selected for the blind measurements: 9 *cfiA*-positive clinical isolates with different imipenem MICs ranging between 0.06 and >32 mg l⁻¹ (different expressions of the metallo- β -lactamase gene) were clearly separated from the 19 *cfiA*-negative isolates. The presence or absence of the selected peaks in all tested strains clearly differentiated the strains belonging to *B. fragilis* division I (*cfiA*-negative) or division II (*cfiA*-positive). These results suggest a realistic method for differentiating division II *B. fragilis* strains (harbouring the *cfiA* gene) and to determine them at a species level at the same time. Although not all *cfiA*-positive *B. fragilis* strains are resistant to carbapenems, they all have the possibility of becoming resistant to this group of antibiotics by acquisition of an appropriate IS element for full expression of the *cfiA* gene, leading to possible treatment failure.

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INTRODUCTION

Bacteroides fragilis, one of the most important species in the *Bacteroides* genus, is the leading anaerobic pathogen in human monobacterial or polymicrobial infections (Aldridge *et al.*, 2003). It is part of the *Cytophaga-Flavobacterium-Bacteroides* group (Gherna & Woese, 1992), which is distant in evolutionary terms from most other bacteria of medical interest. The genus *Bacteroides* appears to be one that evolves rather rapidly (Shah, 1990) and molecular genetic methods have led to novel, highly related species being included in the

genus recently; however, some other species have been removed (Song *et al.*, 2004, 2005; Sakamoto & Benno, 2006). Members of the *Bacteroides* genus, including *B. fragilis*, often exhibit resistance to different drugs, such as β -lactam and β -lactamase inhibitor combinations, clindamycin, fourth-generation fluoroquinolones, metronidazole or carbapenems, used for treatment of infections involving anaerobes. Their correct species identification may be necessary because the resistance to different anti-anaerobic drugs may differ according to the species (Snydman *et al.*, 2007). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to constitute a useful and simple method for the rapid identification of different human pathogenic bacteria, including *Bacteroides* strains, based on the spectrum of constantly expressed high-abundance proteins such as ribosomal proteins or to a less extent the highly prevalent

Abbreviations: IS, insertion sequence; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MSP, main spectra.

A supplementary table showing the characteristic peaks of the *cfiA*-positive and *cfiA*-negative *B. fragilis* strains is available with the online version of this paper.

housekeeping proteins (Bizzini & Greub, 2010; Nagy *et al.*, 2009). Only a few studies have as yet been performed with this method to prove the presence of resistance mechanisms. Cell wall and proteome-related structural alterations reflected in the mass spectra of meticillin-resistant *Staphylococcus aureus* as compared with meticillin-sensitive strains were described some years ago (Edwards-Jones *et al.*, 2000), and a 'proteome shift' in *Candida albicans* that corresponds to its fluconazole MIC measured by conventional methods was reported recently (Marinach *et al.*, 2009).

Despite the fact that *B. fragilis* isolates appear phenotypically homogeneous, DNA–DNA hybridization experiments have proven that they may be divided into two DNA homology groups, I and II, which display 65–70 % intergroup and 80–90 % intragroup similarity (Johnson, 1978; Johnson & Ault, 1978; Gutacker *et al.*, 2000). The two divisions of *B. fragilis* can be distinguished by ribotyping and analysis of the fragment patterns generated by arbitrarily primed PCR or multilocus enzyme electrophoresis (Gutacker *et al.*, 2000, 2002; Podglajen *et al.*, 1995; Ruimy *et al.*, 1996). Two chromosomal cephalosporinase genes have been described in *B. fragilis*. The *cepA* gene encodes a class 2e cephalosporinase (Parker & Smith, 1993). The production of CepA leads to resistance to most β -lactam antibiotics, with the exception of the cephamycins, the carbapenems and the β -lactamase inhibitor combinations. The other chromosomal β -lactamase gene is *cfiA* (also known as *ccrA*), which expresses a class B metallo- β -lactamase that confers resistance in *B. fragilis* to all β -lactam antibiotics, including carbapenems (Rasmussen *et al.*, 1990). For the expression of this gene, special insertion sequence (IS) elements are needed immediately upstream of the *cfiA* gene to act as a promoter (Podglajen *et al.*, 1994). The presence of the *cfiA* gene on the chromosome of randomly selected *B. fragilis* clinical isolates is much more frequent (2.4–6.9 %) than carbapenem resistance (~1 %) detected during resistance surveillances (Nagy, 2010). A silent *cfiA* gene has been shown to be expressed, leading to carbapenem resistance, if the necessary IS element, present in any other part of the chromosome, is inserted in the appropriate upstream region of the gene (Podglajen *et al.*, 1994). The *cfiA* gene can be detected only on the chromosome of *B. fragilis* strains belonging to division II (Gutacker *et al.*, 2000; Podglajen *et al.*, 1995).

The aim of the present study was to use MALDI-TOF MS to distinguish *B. fragilis* strains which harbour the *cfiA* gene from those which do not, thereby providing a possibility for differentiating *B. fragilis* strains belonging to divisions I and II by this method.

METHODS

Isolates. Thirty-eight *B. fragilis* strains were selected from our strain collection for this study; they had known imipenem MICs, and had previously been characterized by PCR and sequencing for the presence of the *cfiA* gene and IS elements in the chromosome (Table 1). All these strains were clinical isolates originating from different resistance surveillance studies carried out locally or on a European level; some of

them originated from the USA. *B. fragilis* NCTC 9343 and TAL3636 were used as control strains belonging to division I and II, respectively. In the second part of the study, the spectra of 145 *B. fragilis* strains previously identified by MALDI-TOF MS (Nagy *et al.*, 2009) were re-evaluated in a search for specific peaks found characteristic of division I or II of *B. fragilis* in the first part of the study. All strains were stored in cryobank vials (Mast Diagnostics) and cultured on Columbia agar base (Oxoid) supplemented with 5 % (v/v) cattle blood, haemin (1 mg l⁻¹) and vitamin K₁ (5 mg l⁻¹) for 24–48 h at 37 °C in an anaerobic chamber (Concept 400; Ruskinn Technology). Imipenem MIC measurements were carried out using Etest (AB Biodisk).

Detection of the *cfiA* gene and IS elements. PCR detection of the *cfiA* gene and the IS elements upstream of the resistance gene (PCR mapping) or in other parts of the chromosome was carried out as previously described (Sóki *et al.*, 2004, 2006).

Sample preparation for MALDI-TOF MS measurement. One colony of each bacterial strain, which had been subcultured for 24 h in an anaerobic environment, was transferred into an Eppendorf vial and carefully suspended in 300 μ l bidistilled water. Ethanol (900 μ l) was added to the suspension, and mixed well. For MALDI-TOF MS analyses the stabilized samples were sent from the Anaerobe Reference Laboratory in Hungary to the Bruker laboratory in Bremen (Germany). Further sample preparation was carried out as described previously (Nagy *et al.*, 2009). In brief, the samples were centrifuged (13 000 g for 2 min), the supernatants were removed and the pellets were dried at room temperature and resuspended in 50 μ l 70 % aqueous formic acid and 50 μ l acetonitrile. After centrifugation (13 000 g for 2 min), 1 μ l of the supernatant was placed onto the MALDI target plate and dried at room temperature, followed by adding 2 μ l MALDI matrix (saturated solution of α -cyano-4-hydroxycinnamic acid in 50 % acetonitrile/2.5 % trifluoroacetic acid).

MALDI-TOF MS measurement. All measurements were performed on a microflex LT MALDI-TOF mass spectrometer in the linear positive ion mode, with a laser frequency of 60 Hz. The mass range was 2000–20 000 Da. For each sample, the sum spectra of 240 single spectra were acquired, in portions of 40 single spectra from 6 different positions on a spot. For each sample, at least 20 separate sum spectra were collected.

Data processing. The mass spectra generated for the analysed bacteria were identified as derived from *B. fragilis* strains by using the MALDI Biotyper 2.0 software and database (version 3.1.1.0., 3740 entries; Bruker Daltonik). Identification was performed with the standard pattern matching approach as recommended by the manufacturer, applying log(score) ≥ 2.0 for species identification.

For the detection of *cfiA*-positive and *cfiA*-negative specific peaks, respectively, mass spectrum sets of well-defined *cfiA*-positive and *cfiA*-negative *B. fragilis* strains were imported in the ClinProTools 2.2 software (Bruker Daltonik). Spectra were normalized and recalibrated using the respective functionalities of the software. Subsequently, group-characteristic peaks and peak shifts were searched for by careful visual investigation of the spectrum sets. In addition, peak variations between the two groups were investigated by using the FlexAnalysis 3.3 software (Bruker Daltonik).

Correlation analysis was performed using the MALDI Biotyper 2.0 software with standard settings (mass range 3000–12 000 Da, resolution 4, eight intervals, autocorrelation off). Twenty different profile spectra were used for each strain in this analysis. Principle component analysis was carried out with the respective ClinProTools 2.2 algorithm for normalized data.

Main spectra (MSP) for the creation of a *cfiA*-positive/*cfiA*-negative differentiating library were calculated from six strains each of which

Table 1. *B. fragilis* strains tested in previous studies for the presence of the *cfiA* gene and selected for the MALDI-TOF MS study

Number	<i>B. fragilis</i>	Origin	Division defined by MALDI-TOF MS profile	PCR detection of <i>cfiA</i> gene	IS element(s)*	Imipenem MIC (mg l ⁻¹)†	Reference
1	TAL3636	USA	II	+	IS942	>32	Rasmussen <i>et al.</i> (1990)
2	NCTC 9343	UK	I	—	—	0.062	Cerdeño-Tárraga <i>et al.</i> (2005)
3	1672	USA	II	+	IS1186, (IS4351)	>32	Sóki <i>et al.</i> (2004)
4	R19811	UK	II	+	IS614B	32	Terhes <i>et al.</i> (2007)
5	O21	Sweden	II	+	IS1186, (IS4351)	>32	Nagy <i>et al.</i> (2011)
6	6712	USA	II	+	IS612B	>32	Sóki <i>et al.</i> (2004)
7	2944	USA	II	+	IS614B	>32	Sóki <i>et al.</i> (2004)
8	CZE60	Czech	II	+	—	16	Sóki <i>et al.</i> (2006)
9	7979	Hungary	II	+	—	16	This study
10	16997	Hungary	II	+	—	16	This study
11	21216	Hungary	II	+	—	8	Sóki <i>et al.</i> (2004)
12	BF8	France	II	+	(IS1186, IS4351)	1	Podglajen <i>et al.</i> (1994)
13	22	Hungary	II	+	—	0.062	Sóki <i>et al.</i> (2000)
14	25877	Hungary	II	+	—	0.125	This study
15	20384	Hungary	II	+	—	0.125	This study
16	72	Hungary	II	+	—	0.25	Sóki <i>et al.</i> (2000)
17	59775	Hungary	I	—	NT	0.25	This study
18	15470	Hungary	I	—	NT	0.25	This study
19	15467	Hungary	I	—	NT	1	This study
20	15361	Hungary	I	—	NT	0.5	This study
21	72232	Hungary	I	—	NT	0.5	This study
22	17797	Hungary	I	—	NT	0.25	This study
23	5269	Hungary	I	—	NT	4	This study
24	P35	Hungary	I	—	NT	0.25	This study
25	8887	Hungary	I	—	NT	2	Nagy <i>et al.</i> (2011)
26	13859	Hungary	I	—	NT	0.25	Nagy <i>et al.</i> (2011)
27	12790	Hungary	I	—	NT	1	Nagy <i>et al.</i> (2011)
28	14850	Hungary	I	—	NT	0.25	Nagy <i>et al.</i> (2011)
29	14285	Hungary	I	—	NT	0.5	Nagy <i>et al.</i> (2011)
30	14176	Hungary	I	—	NT	1	Nagy <i>et al.</i> (2011)
31	AN1040	Sweden	I	—	NT	0.25	Nagy <i>et al.</i> (2011)
32	T1	Turkey	I	—	NT	<0.125	Nagy <i>et al.</i> (2011)
33	T2	Turkey	I	—	NT	<0.125	Nagy <i>et al.</i> (2011)
34	G2	Greece	I	—	NT	0.5	Nagy <i>et al.</i> (2011)
35	DE1	Germany	I	—	NT	<0.125	Nagy <i>et al.</i> (2011)
36	DE2	Germany	I	—	NT	<0.125	Nagy <i>et al.</i> (2011)
37	53156	Finland	I	—	NT	<0.125	Nagy <i>et al.</i> (2011)
38	NL8	The Netherlands	I	—	NT	0.125	Nagy <i>et al.</i> (2011)
39	NL9	The Netherlands	I	—	NT	<0.125	Nagy <i>et al.</i> (2011)
40	638R	USA	I	—	NT	0.5	Sóki <i>et al.</i> (2000)

*IS elements upstream of the *cfiA* genes are indicated. IS elements found by IS PCR, but not upstream of the *cfiA* genes, are shown in parentheses. NT, Not tested.

†Breakpoints for imipenem: susceptible, ≤ 4 mg l⁻¹; resistant, ≥ 16 mg l⁻¹.

had previously been demonstrated to belong to division II or division I. This resulted in a library containing two reference entries: one specific for *cfiA*-positive and one for *cfiA*-negative *B. fragilis* strains. MSP calculations relating to the *cfiA*-positive/*cfiA*-negative library and pattern matching against the created library were performed by using MALDI Biotyper 2.0 with standard settings.

RESULTS

Twelve *B. fragilis* strains (six *cfiA*-positive and six *cfiA*-negative) were selected for the first measurement. Besides the two control strains (NCTC 9343, *cfiA*-negative strain; and TAL3636, *cfiA*-positive strain), ten clinical isolates were

involved. All isolates had previously been identified as *B. fragilis* and investigated in detail for the presence of the *cfiA* gene, the IS elements and the level of imipenem resistance. After the routine measurements, microflex LT MALDI-TOF MS differences were looked for. Figs 1 and 2 show characteristic differences in the interval 4000–5500 Da, visualized by the gel/stack view in ClinProTools with Flexanalysis software. A further 28 strains were selected for the blind measurements: 9 further *cfiA*-positive clinical isolates with different imipenem MICs (different expressions of the metallo- β -lactamase gene) ranging between 0.06 and >32 mg l⁻¹, and 19 *cfiA*-negative isolates with imipenem MICs between <0.125 and 0.5 mg l⁻¹, previously selected from our culture collection and tested for possible silent carriage of the gene. During the two measurements, the presence or absence of the selected peaks in the MALDI-TOF spectrum between 4600 and 10 600 allowed a 100 % distinction between the strains belonging to *B. fragilis* division I (*cfiA*-negative) or division II (*cfiA*-positive) (Supplementary Table S1 in JMM Online).

In a previous collaborative study (Nagy *et al.*, 2009), 277 clinical *Bacteroides* isolates were identified at a species level by MALDI-TOF MS. From among these, the spectra of 145 *B. fragilis* isolates with a high log(score) (≥ 2.5) were re-evaluated and the characteristic peaks defined earlier for

division II were looked for. Nine isolates (6.2 %) belonged to *B. fragilis* division II (Table 2). All nine isolates were *cfiA*-positive, with imipenem MICs varying between <0.125 and >32 mg l⁻¹. Even isolates with silent carriage of the *cfiA* gene were detected by this method. Two of the characteristic peaks (4826 and 9649 Da) were found in all nine strains and a third one (9375 Da) was present in eight of the nine strains.

For further statistical analysis, composite correlation index analysis was performed with profile spectra from the initial six *cfiA*-positive strains and six *cfiA*-negative strains. The correlation between the different *cfiA*-positive strains was found to be 0.84–0.97, while that in the *cfiA*-negative group was 0.86–0.98. In contrast with these high correlations within the two groups, the correlation indices calculated for strains from different groups (i.e. *cfiA*-positive vs *cfiA*-negative) were only 0.45–0.62, confirming the clear spectral difference between strains harbouring or not the *cfiA* gene. Principle component analysis performed with the initial 12 *B. fragilis* strains also resulted in a clear separation of two clusters, consisting of *cfiA*-positive and *cfiA*-negative strains, respectively.

To investigate the possibility of predicting *cfiA*-negative and *cfiA*-positive *B. fragilis* strains with the MALDI Biotyper pattern matching approach, a small library containing only two reference spectra (MSP), one specific for *cfiA*-negative and one for *cfiA*-positive strains, was calculated from the

Fig. 1. Characteristic differences in the MS peaks in the interval 4000–5500 Da between the strains harbouring the *cfiA* gene and those which do not, visualized by the gel/stack view. Peak shifts can be observed from *cfiA*-negative to *cfiA*-positive strains: 4711 Da→4688 Da, 4817 Da→4826 Da, 5017 Da→5002 Da, 5204 Da→5189 Da and 5268 Da→5282 Da.

Fig. 2. Example of the clear differences in the MS peaks in the interval 4650–4850 Da between the *cfiA*-positive and -negative strains. (a) Visualized by the gel/stack view. (b) Visualized by the mass spectra. Peaks are shifted from 4711 Da and 4817 Da (*cfiA*-negative) to 4688 Da and 4826 Da (*cfiA*-positive), respectively.

B. fragilis division I and division II strains from the first test set. This library was challenged with spectra of the 9 further *cfiA*-positive and 19 randomly selected *cfiA*-negative strains (three spectra per strain). In all cases, the MALDI Biotyper software classified the *B. fragilis* strains correctly, indicating the suitability of such an approach for the classification of divisions I and II of *B. fragilis* (data not shown).

DISCUSSION

Since the introduction of MALDI-TOF MS for pure microbiological applications, it has evolved from an experimental tool to a technology with significant benefit for routine as well as for scientific investigations. It is currently applied especially in clinical microbiology for the identification of various bacteria or fungi at a species or subspecies level

Table 2. *B. fragilis* strains identified at a species level in a previous study by MALDI-TOF MS and selected for the current study according to the presence of the characteristic peaks for division II

<i>B. fragilis</i>	Characteristic MS peaks for division II*	<i>cfiA</i> (PCR)	IS upstream of the gene	Imipenem MIC (mg l ⁻¹)
A-20	4826, 9375, 9649	+	—	<0.125
Ba-16	4826, 9375, 9649	+	—	<0.125
Bb-15	4826, 7321, 9375, 9649	+	—	2
Bb-32	4826, 7321, 9375, 9649	+	—	2
Be-3	4826, 7321, 9375, 9649	+	—	0.5
Bm-2	4826, 7321, 9375, 9649, 4688, 5002, 5189	+	—	1
Fr-19	4826, 7321, 9649	+	—	1
Nl-3	4688, 4826, 5002, 5189, 7321, 9375, 9649	+	IS614-like	>32
Nl-1	4688, 4826, 5002, 5189, 7321, 9375, 9649	+	—	2

*Characteristic peaks found in all nine strains (4826 and 9649 Da) or in eight of the nine strains (9375 Da) are in bold.

after a positive culture, as it requires very limited time for sample preparation and measurement (Bizzini & Greub, 2010; Nagy *et al.*, 2009; Murray, 2010). At present, very little information is available on the use of this technique for the direct identification of pathogens from clinical samples with or without preculturing (Drancourt, 2010) or for determination of the antibiotic resistance of isolated pathogens (Edwards-Jones *et al.*, 2000; Marinach *et al.*, 2009).

The results of the present study demonstrate the applicability of MALDI-TOF MS for both the subtyping and the detection of the presence of an important resistance mechanism of *B. fragilis*, a frequently isolated anaerobic pathogen. With the successful differentiation of *B. fragilis* strains belonging to divisions I and II at the same time as their identification at a species level by MALDI-TOF MS, it is possible to predict the presence of an important resistance phenotype with respect to carbapenems, one of the most important group of antimicrobials used in treating mixed infections. The carbapenem resistance of *B. fragilis* is due to a species-specific metallo- β -lactamase, which is encoded by the *cfiA* (*ccrA*) gene of the organism. Almost 100 % of the carbapenem-resistant *Bacteroides* strains are *cfiA*-positive *B. fragilis* isolates. The recognition that *B. fragilis* can be subgrouped into two divisions dates back to the late 1970s, when Johnson & Ault (1978) demonstrated differences in DNA-DNA homology rates not only between the known *Bacteroides* species, but also between two groups of strains of *B. fragilis* subsp. *fragilis* (now *B. fragilis*). Cloning and sequencing of the metallo- β -lactamase gene allowed screening of the *B. fragilis* population, which demonstrated that there are strains that are not imipenem-resistant despite harbouring the *cfiA* resistance gene; in the case of strains with high-level resistances to imipenem, an activator IS element is needed to insert upstream of the *cfiA* gene in normal flora, and clinical or experimental isolates (Podglajen *et al.*, 1994; S3ki *et al.*, 2000, 2004). Further investigations revealed that the *cfiA*-positive strains belong only to division II, and the *cfiA*-positive and -negative strains can be differentiated by various molecular typing methods (Gutacker *et al.*, 2000; Podglajen *et al.*, 1995; Terhes *et al.*, 2007). The finding that the genome and the most important housekeeping genes and rRNA gene sequences differentiate the two divisions reflects the fact that the differences emerged during the early evolution of the species. Through the use of MALDI-TOF MS to identify ribosomal proteins in the present study, the two divisions of *B. fragilis* can be differentiated in a relatively cheap and up-to-date manner. During the time of publication of our results, Wybo *et al.* (2011) using MALDI Biotyper showed through preparing a dendrogram or using the composite correlation index the clear separation of *cfiA*-positive ($n=41$) and *cfiA*-negative ($n=207$) *B. fragilis* strains from their collection.

As concerns the *cfiA*-positive *B. fragilis* isolates selected for our study, the expression of their resistance and genetic

background had previously been investigated in detail: (i) phenotypically resistant strains ($\text{MIC} \geq 16 \text{ mg l}^{-1}$) with IS elements in the upstream region of the *cfiA* genes; (ii) phenotypically resistant strains ($\text{MIC} \geq 16 \text{ mg l}^{-1}$) or with elevated imipenem MICs (1 and 8 mg l^{-1}), but without IS elements upstream of the *cfiA* genes; and (iii) phenotypically fully susceptible strains ($\text{MIC} \leq 0.25 \text{ mg l}^{-1}$) without IS elements upstream of the *cfiA* genes. These three groups of strains could not be distinguished by MALDI-TOF MS, which confirms that more significant genetic changes than merely the movement of IS elements in the genome are responsible for the differences in the MS spectrum. The second group of *cfiA*-positive isolates mentioned above has not yet been sufficiently well characterized as regards the exact expression mechanisms, but our investigations demonstrated that these strains are heterogeneously resistant to carbapenems and may utilize a weak self-promoter of the *cfiA* genes (Rasmussen *et al.*, 1990; Podglajen *et al.*, 1994; our unpublished results).

Recent results have demonstrated that the *cfiA* genes in some *B. fragilis* strains belonging to division II are located in a contiguous sequence with other unrelated genes that replace a portion of a chromosomal segment in the sequenced genome of *B. fragilis* NCTC 9343 belonging to division I (Thompson & Malamy, 1990). However, it has been shown that the *cfiA* genes may be present in multiple copies in the genomes in some strains (Podglajen *et al.*, 1994; our unpublished results). Our explanation for these phenomena is that the chromosomal segment carrying the *cfiA* gene and the accompanying genes forms a genetic element which can be mobile within a strain, but whose transfer between the two divisions is restricted.

Although not all *cfiA*-positive *B. fragilis* strains are resistant to carbapenems, they all have the potential to become resistant to this important group of antibiotics by acquisition of an appropriate IS element for full expression of the *cfiA* gene. Confirmation by MALDI-TOF MS of the separation of the *B. fragilis* genomic divisions at the same time as the species determination could be useful in clinical microbiology laboratories for the detection of resistance to this important antibiotic in *B. fragilis*.

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