Research Square

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Higher prevalence of multi-antimicrobial resistant Bacteroides spp. strains isolated at a tertiary teaching hospital, China

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Abstract

Background: The antimicrobial resistance of Bacteroides spp. isolates is reported to be increasing among different districts around the world, and few reports found that multi-antimicrobial resistant strains emerged. However, less is known about the prevalence of multi-antimicrobial resistant isolates in China, especially for carbapenem resistance.

Methods: Bacteroides spp. clinical strains were isolated from inpatients at a 3000 bed tertiary teaching hospital , and were identified by MALDI-TOF MS and VITEK-2 anaerobes and corynebacterium (ANC) card. Broth microdilution method was employed to detect the antimicrobial sensitivities of Bacteroides spp. isolates and PCR method was used to detect the resistance genes, including cfxA, cepA, cfiA, ermF and nim. The upstream insertion sequence (IS) element of cfiA gene was further detected and verified.

Results: Among 115 Bacteroides spp. strains enrolled in this study, 80 isolates were Bacteroides fragilis and 35 isolates were non-Bacteroides fragilis. The total resistance rates of 115 Bacteroides spp. isolates to ampicillin/sulbactam, amoxicillin/clavulanic, cefoxitin, piperacillin, piperacillin/tazobactam, moxifloxacin, clindamycin, metronidazole, imipenem and meropenem were 22.6%, 19.6%, 3.5%, 27.8%, 8.7%, 16.5%, 80.0%, 5.2%, 13.9% and 13.9% , respectively. Except cefoxitin and moxifloxacin, the resistance rates of B. fragilis isolates to the above antibiotics were all higher than those of non-B. fragilis isolates. The positive rates of carbapenem resistance gene cfiA were 38.9% and 8.6% for B. fragilis and non-B. fragilis isolates, respectively. For 15 carbapenem resistant B. fragilis isolates, the co-carrying rates of carbapenem resistance gene cfiA and its upstream IS element were 86.7% (13/15).

Conclusions: The overall resistance rates of Bacteroides spp. isolates toward multiple antibiotics were at a higher level, especially for B. fragilis. CfiA gene carrying rate among B. fragilis isolates was high up to 38.9% and its mediated carbapenem resistance was a major resistance mechanism for B. fragilis. The findings of this study imply that the actual resistance tendency of Bacteroides spp. may be underestimated and need to be given more attention to.

Introduction

Bacteroides spp. is a kind of common anaerobes inhabiting the intestines of humans and is the most common anaerobes recovered from various infections, such as intra-abdominal infection, foot ulcer and bloodstream infection [1, 2, 3]. In recent years, multi-drug resistant *Bacteroides fragilis* emerged in several areas around the world [4, 5, 6], but resistance features varied greatly among different areas [7].

In treating infection with *Bacteroides* spp., metronidazole, clindamycin, some β-lactams and fluoroquinolones were routinely prescribed. Based on relevant literature, resistance of *Bacteroides* spp. isolates to these kinds of antibiotics was related with some specific resistance genes. *nim* gene was found to be closely correlated with metronidazole [8], while the presence of *cfxA* gene was strongly related with cefoxitin resistance [9]. Macrolide-Lincosamide-Streptogramin B resistant determinants, *erm* genes are widely distributed among *Bacteroides* spp. isolates and *ermF* gene is responsible for *B. fragilis*

resistance to clindamycin [7, 10]. The existence of imipenem-resistant *Bacteroides* spp. strains was firstly reported over 3 decades ago [11]. As reported in present studies, the primary mechanism of carbapenem resistance of *B. fragilis* was production of metallo-β-lactamase encoded by the *cfiA* gene [12] and specific upstream insertion sequence (IS) element is required for its expression, which was confirmed by some subsequent investigations [7, 13]. However, owing to lack of popularization of anaerobes culture among Chinese hospitals, less is known about the actual resistance tendency and resistance mechanism of *Bacteroides* spp. clinical isolates.

In this study, we try to detect the resistance features and possible resistance mechanism of *Bacteroides* spp. clinical isolates collected at a 3000-bed tertiary teaching hospital in China from March 2017 to February 2019.

Methods

Anaerobes culture, isolation and identification

From March 2017 and February 2019, all of the anaerobes samples were cultured under anaerobic condition, and anaerobes isolates were collected from clinical samples of patients from Affiliated hospital of Inner Mongolian Medical University and identified with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (microTyper MS, Tianrui, China) and VITEK-2 Compact automated microbiology system (BioMérieux, France) with anaerobes and corynebacterium (ANC) card (BioMérieux, France).

Antibiotics susceptibility test

The minimal inhibitory concentration (MIC) values of 11 antibiotics (ampicillin/sulbactam, amoxicillin/clavulanic acid, imipenem, cefoxitin, meropenem, piperacillin, moxifloxacin, piperacillin/tazobactam, metronidazole, clindamycin, tigecycline) of all the strains were determined using broth microdilution method under anaerobic condition according to the recommendation of Clinical and Laboratory Standard Institute (CLSI) [14] and EUCAST [15]. The following concentrations of the antibiotics were tested: ampicillin/Sulbactam (0.25/0.125-32/16 μg/ml), amoxicillin/calvulanic acid (0.125/0.06-16/8 μg/ml), imipenem (0.25-32 μg/ml), cefoxitin (0.5–64 μg/ml), meropenem (0.25-32 μg/ml), clindamycin (0.125-16 μg/ml), piperacillin (0.5–128 μg/ml), metronidazole (0.25-32 μg/ml), moxifloxacin (0.06-8 μg/ml), piperacillin/tazobactam

(0.5/4–64/4 μg/ml), tigecycline (0.25-32 μg/ml). After 48 hours incubation at 35 °C under anaerobic condition, the antimicrobial MIC values were evaluated based on the breakpoints of CLSI and EUCAST [15] (Table 1). *B. fragilis* ATCC 25285 was applied as the control strain. For the isolates with reduced susceptibility or resistant to carbapenem antibiotics, production of metallo-β-lactamases (MBLs) was detected by EDTA inhibition assay.

Table 1

In vitro activities of 11 antimicrobial agents against 115 Bacteroides spp. isolates

Antimicrobials	MIC (μg/ml)			No. of strains (%)		
	Range	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Bacteroides fragilis (80)						
Ampicillin/sulbactam	0.25/0.1–32/16	4	≥ 32	62.5	10.0	27.5
Amoxicillin/clavulanic acid	0.125/0.06-16/8	2	≥ 16	61.2	5.0	33.8
Cefoxitin	0.5–64	4	32	83.7	3.8	2.5
Piperacillin	0.5–128	8	≥ 64	67.4	3.8	28.8
Piperacillin/tazobactam	0.5/4–64/4	2	≥ 64	88.7	0.0	11.3
Imipenem	0.25-32	2	32	74.9	6.3	18.8
Meropenem	0.25-32	0.5	≥ 32	77.4	3.8	18.8
Clindamycin	0.125-16	≥ 16	≥ 16	12.5	0.0	87.5
Metronidazole	0.25-32	0.5	8	92.5	0.0	7.50
Moxifloxacin	0.06-8	1	8	76.2	7.5	16.3
Tigecycline [a]	0.25-32	1	4	-	-	-
Non-B.fragilis(35)						
Ampicillin/sulbactam	0.25/0.1–32/16	4	32	77.2	11.4	11.4
Amoxicillin/clavulanic acid	0.125/0.06-16/8	2	16	71.4	8.6	20.0
Cefoxitin	0.5–64	8	32	83.3	11.4	5.7
Piperacillin	0.5–128	32	≥ 64	74.3	0.0	25.7
Piperacillin/Tazobactam	0.5/4–64/4	4	8	97.1	0.0	2.9
Imipenem	0.25-32	4	8	82.8	14.3	2.9
Meropenem	0.25-32	0.5	1	97.1	0.0	2.9

a No breakpoint is available.

Antimicrobials	MIC (μg/ml)			No. of strains (%)		
	Range	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Clindamycin	0.125-16	≥ 16	≥ 16	31.4	0.0	62.9
Metronidazole	0.25-32	0.25	1	100	0.0	0.0
Moxifloxacin	0.06-8	0.5	8	82.9	0.0	17.1
Tigecycline [a]	0.25-32	0.5	2	-	-	-
a No breakpoint is available.						

Detection of resistance genes

Total DNA of the isolates were extracted by bacterial DNA extraction kit (Qiagen Ltd, Germany) and the most common antibiotic resistance genes (*cepA, cfxA, cfiA, ermF, nim*) [13] and the insertion sequence (IS) upstream [4] of the *cfiA* gene was amplified. Then the PCR products were analysed with 1.2% agarose gel electrophoresis, and the DNA amplicon of the PCR was purified using the QIA quick Gel Extraction Kit (Qiagen Ltd, Germany). The IS elements were further verified by PCR method with well-known primers [16] and sequenced.

Results

Antibiotic susceptibility test results

The strains tested in this study were isolated from the following specimens: abdominal pus samples (99 cases), cervical canal samples (7 cases), blood samples (5 cases), oral pus samples (2 cases) and skin wound/abscess samples (2 cases). Among 115 *Bacteroides* spp. isolates, *B. fragilis* accounted for 69.6% (80/115), followed by *Bacteroides thetaiotaomicron* (17.4%, 20/115), *Bacteroides ovatus* (3.5%, 4/115), *Bacteroides uniformis* (2.6%, 3/115), *Bacteroides vulgatus* (2.6%, 3/115), *Bacteroides novobacterioides* (2.6%, 3/115), and *Bacteroides distasonis* (1.7%, 2/115). The total resistance rates of 115 *Bacteroides* spp. isolates to ampicillin/sulbactam, amoxicillin/clavulanic□cefoxitin, piperacillin, piperacillin/tazobactam, moxifloxacin, clindamycin, metronidazole, imipenem and meropenem were 22.6%, 19.6%, 3.5%, 27.8%, 8.7%, 16.5%, 80.0%, 5.2%, 13.9% and 13.9%, respectively.

Comparing with non-*B. fragilis* isolates, *B. fragilis* isolates showed higher level of resistance to most of the antibiotics tested in this study, as shown in Table 1. For 80 *B. fragilis* isolates, the resistance rates of 80 *B. fragilis* isolates to both imipenem and meropenem were 18.8% (15/80), and only 1 *B. thetaiotaomicron* isolate was found to be carbapenem resistant. For 26 *Bacteroides* spp. isolates with reduced susceptibility or resistant to imipenem or meropenem, 16 isolates were found to MBL producers,

including 15 isolates of *B. fragilis* and 1 isolates of *B. thetaiotaomicron.* Moreover, MIC_{90} values of *B. fragilis* and non-*B. fragilis* isolates to tigecycline were 4 μg/ml and 2 μg/ml, respectively.

Distribution of resistance genes

Among 115 *Bacteroides* spp. isolates, the positive rates of resistance gene *cfiA, cfxA, cepA, ermF* and *nim* were 29.6% (34/115), 32.2% (37/115), 65.2% (75/115), 89.6% (103/115) and 30.4% (35/115) , respectively. The positive rates of above resistance genes of *B. fragilis* isolates were all significantly higher than those of non-*B. fragilis* isolates, as shown in Fig.1. 31 *B. fragilis* and 4 non-*B.fragilis* isolates were positive with *nim* gene, but only 6 *B. fragilis* isolates showed resistance to metronidazole.

For 15 imipenem resistant *B. fragilis* isolates, *cfiA* gene were all positive and 13 isolates harboured the IS element, which belonged to IS1187 (11 isolates), IS942 (1 isolate) and IS1169 (1 isolate), as shown in table 2. One non-*B. fragilis* isolate was found to be carbapenem resistant, which was positive with *cfiA* gene but no IS element was detected(Fig. 2). Among the 13 isolates with both *cfiA* gene and IS element, BF18 strain had a significant longer IS element (3290bp) than any other IS elements of the other 12 strains and the IS sequence was a inverted repeat segment, while there were two inverted repeats in another normal isolate (BF15) belonged to IS1187.The positional relations between *cfiA* gene and IS sequence within BF18 and BF15 strains was shown in Fig. 3.

Table. 2 Characteristics of 16 carbapanem resistant *Bacteroides* spp. isolates

Isolates	MIC(μg/ml)		EDTA inhibition assay	Resistance genes					
	imipenem	meropenem		cfiA	IS element	cepA	ermF	cfxA	nim
BF09	16	16	+	+	IS1187	+	+	+	-
BF15	32	≥32	+	+	IS 1187	-	+	+	+
BF18	≥32	≥32	+	+	IS 942	+	+	+	+
BF20	16	≥32	+	+	IS 1187	-	-	-	-
BF31	≥32	≥32	+	+	IS 1187	-	+	+	-
BF36	≥32	≥32	+	+	IS 1169	-	+	+	+
BF37	16	16	+	+	IS 1187	-	+	+	+
BF41	16	32	+	+	IS 1187	-	+	-	+
BF43	16	32	+	+	IS 1187	+	+	-	-
BF49	16	32	+	+	IS 1187	-	+	+	-
BF58	≥32	≥32	+	+	None	-	+	-	+
BF60	32	≥32	+	+	None	+	+	-	+
BF67	32	≥32	+	+	IS 1187	-	+	-	+
BF69	16	≥32	+	+	IS 1187	-	+	-	+
BF76	16	32	+	+	IS 1187	-	+	-	-
BT10	16	≥32	+	+	None	-	+	-	-

Note: BF, *Bacteroides fragilis*; BT, *Bacteroides thetaiotaomicron*

Discussion

In recent years, reports of anaerobes resistance features showed an increasing tendency, but few were from China. In this study, 115 *Bacteroides* spp. clinical strains were isolated from a 3000-bed tertiary teaching hospital in China and showed a higher resistance rates to multiple commonly used antibiotics, including several β-lactams, metronidazole, et al. Especially, the resistance rates of *Bacteroides* spp. isolates to carbapenem and metronidazole was at a higher level, and the isolates were mainly *B. fragilis*. It is well known that the mechanism of carbapenem resistance within *B. fragilis* is mianly mediated by *cfiA* gene and the upstream IS element is required. In this study, the positive rate of *cifA* gene of carbapenem resistant *B. fragilis* reached up to 38.9%, which is obviously higher than other reports published recently [7, 17]. It is noteworthy that *B. fragilis* strains with *cfiA* gene can easily converted to be resistant ones by the effects of its upstream IS element [18], which implies that a larger group of patients maybe a potential

reservoir of carbapenem resistant *B. fragilis* producers, who are colonized or infected by *B. fragilis* with silent *cfiA* gene. Since the *B. fragilis* isolates tested in this study were mainly from intra-abdominal samples, intestinal screening of *cfiA* positive *B. fragilis* within a specific group of people seems to be beneficial for better understanding the actual distribution of *cfiA* gene. So far, few reports showed that IS-less activation mechanism were found to be existed at *B. fragilis* isolates with elevated imipenem MICs or imipenem reisistant strains [16]. In this study, two *B. fragilis* isolates and 1 non-*B. fragilis* isolate resistant to carbapenem were found to be *cfiA* positive and IS element was deficient. The potential non-IS resistance mechanism needs to be further investigated. Also, a longer IS element (3290 bp) was detected in a carbapenem resistant *B. fragilis* isolate, which was significantly different with other IS elements observed in this study. For this isolate , the MIC value of imipenem and meropenem were both higher than 32 μg/mL, which may possess a special regulating mechanism in mediating carbapenem resistance.

Besides carbapenem antibiotics, the resistance rates of *B. fragilis* isolates to several other β-lactam antibiotics were all higher than those of non-*B.fragilis* isolates, including ampicillin/sulbactam, amoxicillin/clavulanic acid, peperacillin and peperacillin/tazobactam. Consistent with resistance phenotype, the prevalence of *cepA* and *cfxA* genes among *B. fragilis* isolates were significantly higher than those of non-*B. fragilis* isolates. Veloo ACM et al [5] reported that *cfiA* and *cepA* genes together were responsible for amoxicillin resistance, but none of the *B. fragilis* isolates harboured the both genes in this study. However, resistance of amoxicillin was not detected and a total of 20 *B. fragilis* isolates harboured both *cfiA* and *cepA* in this study. It is noteworthy that 9 non-*B. fragilis* isolates harboured *cepA*, which was obviously different with those reported in some previous reports [5, 19, 20]. Kierzkowska M et al [9] reported that in *B. fragilis*, cefoxitin phenotypic resistance was strongly correlated with *cfxA* gene expression. However, only 2 *B. fragilis* isolates and 2 non-*B. fragilis* isolates were resistant to cefoxitin and none of the isolates harboured *cfxA* gene in this study. Owing to the limitation of the number of isolates, the correlation between cefoxitin resistance and expression of *cfxA* gene can't be confirmed.

The resistance rate of *B. fragilis* to metronidazole in this study was 7.5%, which is obviously higher than previous reports [5, 6, 7, 9, 21, 22], and no metronidazole resistant non-*B. fragilis* isolate was detected. To date, the mechanism of metronidazole resistance is still not well-known, and it is reported that *nim* genes are closely correlated with metronidazole resistance [23]. The positive rate of *nim* gene in this study was 38.9% and 11.4% for *B. fragilis* and non-*B. fragilis* isolates, respectively, but the correlation between metronidazole resistance and expression of *nim* gene was not observed. For 6 metronidazole resistant *B. fragilis* isolates, only 3 isolates were positive with *nim* gene.

In this study, total resistance rate of *B. fragilis* isolates to clindamycin reached up to 87.5%, significantly higher than that reported by Fernández-Canigia L et al [6] and Justesen US et al [24]. It is well known that *erm*(A-F) are widely distributed in *Bacteroides* spp. strains and are responsible for macrolide-lincosamide-streptogramin B resistance in *B. fragilis* [25]. In the present study, the positive rate of *ermF* gene (94.7%) was comparable with the phenotypic resistance rate (87.5%), which suggests that *ermF* gene may be mainly responsible for *B. fragilis* resistance in this study.

The resistance rate to moxifloxacin was 16.5% and is significantly lower than that observed in another recent study from China [7], but there is no obvious difference between *B. fragilis* isolates and non-*B. fragilis* isolates. It is reported that fluoroquinolone resistance may be correlated with multiple mechanism, including mutation of quinolone resistance determining region of the genes of gyrase and/or topoisomerase IV, as well as increased efflux [10, 26]. However, the related resistance genes were not detected and exploration of the resistance mechanism didn't fall the scope of this study.

For tigecycline, the MIC_{50} and MIC_{90} for both *B. fragilis* and non-*B. fragilis* isolates in this study were all higher than those reported in a previous study [6]. Since there is no available breakpoint for tigecycline, it is not feasible to assess its clinical significance until now. Also, the antibiotics susceptibility testing method used in this study was broth microdilution method, the results need to be further verified with agar dilution method in subsequent investigation.

Conclusions

The total resistance rates of *Bacteroides fragilis* clinical isolates to several commonly used antibiotics were higher compared with some other reports recently published, which implies that the actual resistance tendency of anaerobes may be underestimated. A limitation of this study is that the isolates were obtained only from one hospital, and a multi-centre study with a large sample size should be carried out to further investigate the actual resistance feature of anaerobes, especially for carbapenem resistant *B. fragilis* isolates.

Abbreviations

MIC
Minimum inhibitory concentration;
MIC_{50}
50% minimun inhibitory concentration
MIC_{90}
90% minimun inhibitory concentration

Declarations

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Authors contributions

Yanyan Wang and Wenqi Zheng are responsible for antibiotics susceptibility test and resistance gene detection. Yingying Lv and Huimin Shen are responsbile for isolates isolation and identification.Junrui Wang is responsible for data analysis and manuscript writing.

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Availability of data and materials

Available at request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study is exempt from ethical consent according to the local ethical guidelines.

Competing interests

The authors declare that they have no competing interests.

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Figures

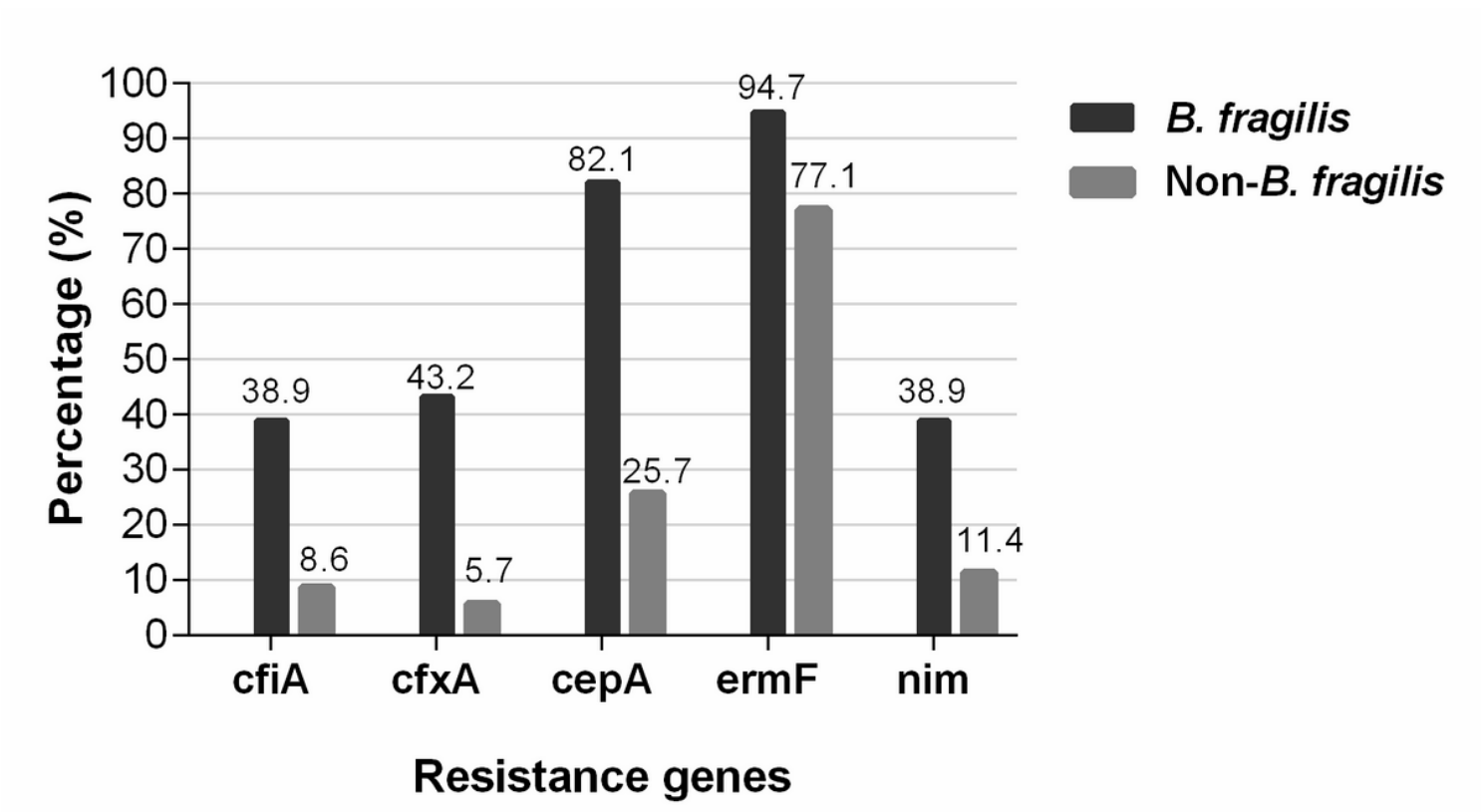

Figure 1

Prevalence of resistance genes among B. fragilis and non-B. fragilis isolates

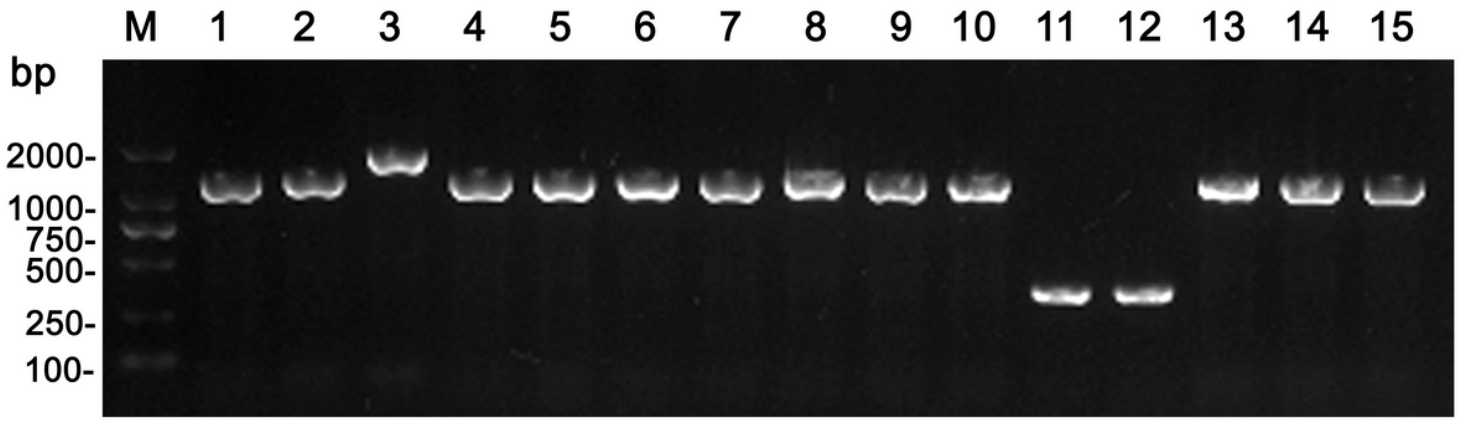

Figure 2

PCR results of cfiA and IS element of 15 carbapenem resistant B. fragilis isolates Note: 1, BF09; 2, BF15; 3, BF18; 4, BF20; 5, BF31; 6, BF36; 7, BF37; 8, BF41; 9, BF43; 10, BF49; 11, BF58; 12, BF60; 13, BF67; 14, BF69; 15, BF76

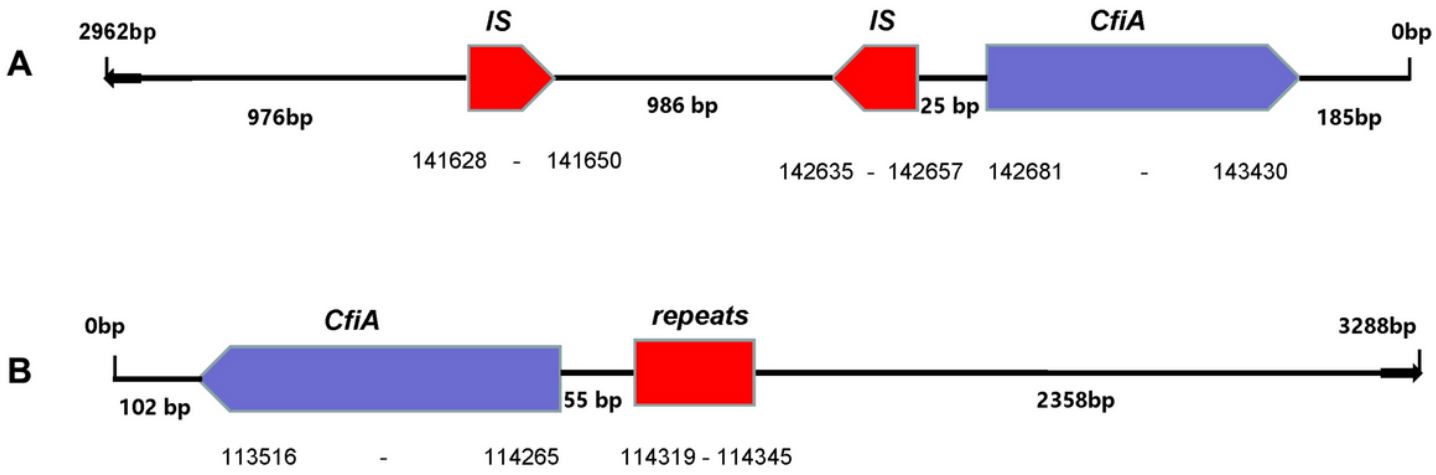

Figure 3

IS element analysis of BF15 and BF18 isolates