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THE FIRST CHARACTERIZED CARBAPENEM-RESISTANT *BACTEROIDES FRAGILIS* STRAIN FROM CROATIA AND THE CASE STUDY FOR IT

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An imipenem-resistant *Bacteroides fragilis* strain was isolated from the blood of a 72-year-old male patient with a urinary bladder tumor in Osijek, Croatia. This strain was also resistant to ampicillin, piperacillin/tazobactam, cefoxitin, clindamycin, tetracycline, and harbored *cfiA, ermF*, and *tetQ* genes where the high-level expression of the *cfiA* carbapenem-resistant gene was driven by an IS*1187* element. Interestingly, despite the carbapenem-resistant feature of the *B. fragilis* from blood, the patient relatively easily recovered from the bacteremia. It was the first characterized imipenem-resistant *B. fragilis* isolate with its case report from Croatia, which confirmed the appearance of carbapenem-resistant *B. fragilis* strains, that continues worldwide with low incidence and the molecular characteristics vary temporally and geographically.

Keywords: *B. fragilis*, carbapenem resistance, ertapenem, imipenem, IS*1187*

Introduction

Bacteroides fragilis is the most commonly isolated opportunistic anaerobic pathogen, which is also a member of the normal microbiota. It comprizes 60%–80% of the isolated anaerobic pathogens, but gives a Bacteroides count of only 0.5%–1% in the gut. The infections caused vary from mild to life-threatening conditions, such as diarrhea, abdominal abscessi, other superficial soft-tissue infections, or sepsis. As a member of its parent genus, the *Bacteroides*, it is

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highly resistant to antibiotics regarding both the prevalence and the number of resistance mechanisms. *B. fragilis* is often resistant to regular β-lactams (penicillins and cephalosporins) (about 99%) and tetracyclines (about 70%). It may be resistant (about 10%–30%) to cephamycins (cefoxitin), clindamycin, or moxifloxacin. The best available antibiotics are β-lactam/β-lactamase inhibitor combinations, carbapenems, metronidazole, or tigecycline. Its carbapenem resistance is mediated by a metallo-β-lactamase, which is coded by the *cfiA* gene [1]. This gene is present to a significant extent in the *B. fragilis* population, of which a distinct subgroup of the species can be identified by molecular methods. To be highly carbapenem-resistant, an insertion sequence (IS) element is necessary whose role is to provide a strong promoter for expression [2]. As we mentioned, the prevalence of true carbapenem-resistant *B. fragilis* strains is low, but such strains might still pose a serious treatment problem, if it is unrecognized [3]. The involvement of IS elements in driving the phenotypic expression of other antibiotic-resistant genes [*cepA* – penicillin, cephalosprorin, *cfxA* – cephamycin, *ermF* – clindamycin, and *nim* – metronidazole] different from *cfiA* is well known for clinical *Bacteroides s*trains [1].

In this report, we describe a case and characterization of an imipenem-resistant *B. fragilis* strain isolated at the Institute of Public Health for Osijek-Baranja County, Osijek, Croatia. Resistance to carbapenems among *Bacteroides* spp. isolates was previously reported in Croatia by Novak et al. [4], but the mechanisms of resistance were not characterized or described.

Materials and Methods

The *Bacteroides* strains ($n = 15$) in this study were isolated from clinical specimens at the Institute of Public Health for Osijek Baranja County, Osijek, Croatia, they were identified and cultured by routine methods [5], and their diagnostic antibiotic susceptibility measurements were performed by the ATB AN® System (bioMerieux, France). For long-term storage of the strains, their brain–heart infusion cultures containing 20% glycerol were stored at −70 °C. After sending the strains to the Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary and ESCMID Study Group on Anaerobic Infections (ESGAI) cooperation, their species identities and associated genetic division of *B. fragilis* were analyzed by MALDI-TOF MS (Microflex, Bruker Daltonics, Bremen, Germany) [6]. Antibiotic susceptibility confirmation of *B. fragilis* O4/13 (the only imipenem-resistant strain belonging to genetic Division II) was performed by the E-test method, as described by bioMeriux. Susceptibility/resistance categorization was carried out according to the EUCAST (http://www.eucast.org/clinical_breakpoints/) recommendations and

since they are not available for moxifloxacin and tigecycline, we used the data from Eitel et al. [7].

The presence of antibiotic-resistant genes (*cepA, cfxA, cfiA, nim, ermF, bexA, tetQ, tetX*, and *tetX1*) and their activation IS elements was detected as described earlier [8]. In brief, DNA templates were prepared by the boiling method and RT-PCR was carried out in 10 μl final volumes using 5 μl of Brilliant II RT-PCR master mix with SybrGREEN and ROX dyes (Agilent, Santa Clara, CA, USA), 0.7 μM of primers, and 1 μl of DNA templates with the primers and cycling conditions described earlier, using a StepOne RT-PCR instrument (LifeTechnologies). The harboring of the enterotoxin gene (*bft*) was detected in a similar way as the antibiotic-resistant genes as described in [9]. The plasmid content was examined using the Birnboim–Doly alkalic detergent lysis method [9, 10].

Results

In the period 2013–2016, antibiotic susceptibility testing using commercially available microdilution test ATB AN® for 15 *B. fragilis* group strains was carried out at the Institute of Public Health for Osijek-Baranja County and one, namely *B. fragilis* O4/13, proved to be imipenem-resistant. These strains were sent to the Institute of Clinical Microbiology, University of Szeged in an ESGAI cooperation. Their species identifications were confirmed by MALDI-TOF MS and this strain also turned out to belong to Division II of *B. fragilis* by the MALDI-TOF MS project analysis (the remaining ones belonged to Division I). The case for this isolate is described as follows. The male patient (born in 1941) underwent an operation to remove a urinary bladder tumor (Dg: *Adenocarcinoma vesicae urinariae*) in January 2013 when a nephrostomal catheter along with permanent urinary catheter was inserted. In the following months, he regularly visited his urology specialist. During a checkup in August, he complained of a pus discharge from his nephrostomal catheter and was admitted to the infectious diseases ward at the University Hospital Osijek, Osijek, Croatia for further treatment. On admission, he was afebrile, and his blood pressure was 110/70 mmHg, and ECG without any abnormalities (sinus rhythm, heart rate: 92 beats/min). The laboratory findings were as follows: 86.7 mg/L C-reactive protein (CRP), 10.9×10^9/L WBC, 2.37×10^{12}/L RBC, 62 g/L hemoglobin, 0.189 hematocrit, 293×10^9/L platelets, 25.5 mol/L urea, 425 μmol/L creatinine, 136 mmol/L Na, 5.2 mmol/L K, 14 U/L aspartate aminotransferase (glutamate oxaloacetate transaminase), 27 U/L alanine aminotransferase (glutamate pyruvate transaminase), and 51 U/L gamma glutamyl transferase. From a urine sample collected using the left nephrostomal catheter, an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* was isolated. Since the patient was

not allergic to antibiotics, along with replacement of the indwelling catheter therapy with ertapenem 1 × 1 g was introduced. On the fourth day of hospitalization, the patient experienced a peak in elevated body temperature (38 °C), so blood was collected for a blood culture set. From the inoculated anaerobic bottle, the *B. fragilis* was isolated and the aerobic bottle remained sterile. The ertapenem therapy was continued in spite of this. It is worth adding that the patient also had an *anus praeter* placed following radical surgery due to a malignant growth in the rectum 5 years earlier. After 7 days of ertapenem therapy and two transfusions (RBC concentrate, blood group 0, Rh-positive), the patient's health improved. At this point, his laboratory findings were as follows: 27.5 mg/L CRP, 11.1×10^9/L WBC, 3.12/L RBC, 92 g/L hemoglobin, 0.277 hematocrit, 347×10^9/L platelets, 19 mmol/L urea, 329 μmol/L creatinine, 144 mmol/L Na, 4.8 mmol/L K, and repeated culture of urine sample remained sterile. After resolving the acute urinary tract infection (*K. pneumoniae*) by ertapenem and the bacteremia probably due to the blood transfusion, he was transferred to the oncology department for continuing further treatment of developed metastatic processes. The treatment with capecitabin and irinotecan was continued and the patient was released and sent home with the recommendation of regular oncology checkups.

The *B. fragilis* O4/13 strain was further characterized by microbiological and molecular methods reported earlier. The data obtained by these methods are shown in Table I. The strain was resistant to ampicillin, piperacillin/tazobactam, cefoxitin, imipenem, clindamycin and tetracycline, intermediate-resistant to amoxicillin/clavulanate and susceptible to metronidazole, moxifloxacin, tigecycline, and chloramphenicol. From these results, it was classed as a multidrug-resistant (MDR) isolate, since it exhibited resistance to three classes of antibiotics (β-lactams, lincosamide, and tetracycline). However, this distinction is not so clear-cut, since the penicillin/cephalosporin, clindamycin, and tetracycline resistances are fairly frequent among *Bacteroides* and *Parabacteroides* strains. We detected the *cfiA* gene with an upstream, activating IS element (IS*1187*) explaining the β-lactam resistances [11], an *ermF* gene and the common *tetQ* gene that might have caused the tetracycline resistance. The clindamycin resistance might have been caused by the *ermF* gene, but we did not find the IS*4351* element in its upstream region (Table I). However, such instances are known where the clindamycin resistance can be explained by the *ermF* gene without the well-known IS*4351* [8]. No enterotoxin (fragilysin), *bft*, gene was detected in the strain and the only plasmid that was detected in it was a 4.1 kb cryptic plasmid.

Discussion

In this study, we described a carbapenem-resistant *B. fragilis* strain isolated in Osijek, Croatia, with the description of its clinical case and additionally we also

Table I. Antibiotic resistance data and the corresponding molecular characteristics of *B. fragilis* O4/13

Resistance to antibiotics	Antibiotic MIC (μg/ml)	Amp **>256**	AMC 8	Ptc **64**	FX **64**	IP **>32**		CLI **>256**		MTZ 0.064	MOX 0.125	Tet **16**	TGC 0.25	CHL 8
Antibiotic-resistant genes or IS elements	Gene or IS		*cepA*		*cfxA*	*cfiA*	*cfiA*-IS[a]	*ermF*	IS*4351*	*nim*	*bexA*	*tetQ*	*tetX*/*tetX1*	NA
	Presence of genes and IS element		−		−	+	IS*1187*	+	−	−	−	+	−	NA

Note: The values in the resistance ranges are represented in bold. The abbreviations denote the following antibiotics. Amp: ampicillin; AMC: amoxicillin/clavulanic acid; Ptc: piperacillin/tazobactam; IP: imipenem; CLI: clindamycin; MTZ: metronidazole; MOX: moxifloxacin; Tet: tetracycline; TGC: tigecycline; CHL: chloramphenicol; MIC: minimum inhibitory concentration; FX: cefoxitin; NA: not applicable.

[a]The IS element upregulating the *cfiA* gene obtained after the PCR amplification and sequencing of the upstream region of *cfiA*.

reported its microbiological and molecular biological characterizations. In this concern, we can add that true/phenotypic carbapenem resistance is still very rare among *Bacteroides*. However, there were numerous batch studies that reported this kind of resistance to several strains in France, Japan, USA, Hungary, Kuwait, all over Europe, Korea, Denmark, and Turkey [2, 12–17]. Several other studies reported unique strains that were isolated separately [18, 19]. For these latter strains, we also know the clinical backgrounds that were often critical to the patients harboring them. Usually, their MDR phenotypes were the major obstacles in treating the patients empirically [3]. Although the present strain isolated in Osijek, Croatia, could be regarded as MDR, the patient fortunately recovered from it with only a little help and the strain did not harbor any other significant resistance type except for some resistance to clindamycin and tetracycline. Overall, its carbapenem-resistance mechanism is consistent with our knowledge, *cfiA* with an IS element (IS*1187)* upstream, and it also indicates the spread of IS*1187*, but the high clindamycin resistance (MIC > 256 μg/ml, *ermF* without IS*4351*) requires further investigation. The characterization of this imipenem-resistant *B. fragilis* strain from Osijek, Croatia, has contributed to our knowledge or at least it warns us about the occurrence, importance, and biological variability of carbapenem-resistant *B. fragilis* isolates.

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Conflict of Interest

None.

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