Case Report

Multidrug-resistant *Bacteroides fragilis* group on the rise in Europe?

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We report a case of multidrug-resistance (MDR) in a strain of *Bacteroides fragilis* from a blood culture and abdominal fluid in a Danish patient. The patient had not been travelling for several years and had not received antibiotics prior to the present case. We also summarize the cases that have been reported to date of MDR *B. fragilis* group in Europe. As far as we know, a case like this with MDR *B. fragilis* has not been described in Scandinavia before.

Introduction

Bacteroides fragilis group strains are important opportunistic pathogens and the most frequently isolated anaerobic bacteria from patients with intra-abdominal infections, deep tissue infections, abscesses and bacteraemia (Löfmark *et al.*, 2010; Nagy, 2010). It is known that recently there has been an increase in resistance to antibiotics commonly used for anaerobic bacteria (Nagy *et al.*, 2011), and also an increase in resistance to carbapenems, and in some cases to metronidazole. Recently, two studies have demonstrated that alarming numbers of *B. fragilis* group isolates harbour the *cfiA* gene, with 27 % of *Bacteroides* species from a Turkish university hospital and 7.4 % of *B. fragilis* isolates from a Belgian multi-centre survey possessing *cfiA* (Toprak *et al.*, 2012; Wybo *et al.*, 2011). Although the presence of the *cfiA* gene does not equate to resistance to carbapenems, this development is of some concern.

Case report

An 84-year-old male was diagnosed with colon cancer in July 2010. In September 2010 he was admitted for laparoscopic hemicolectomy. After the operation he had complications with bleeding from several drains in his abdomen. Six days after the operation his condition worsened, with signs of sepsis. Antibiotic treatment with meropenem 1 g three times a day and metronidazole 500 mg three times a day was initiated, and he was transferred to the intensive care unit (ICU).

At the ICU the patient experienced bacteraemia and fungaemia with *Enterococcus faecium*, *Candida glabrata* and *Candida albicans*; this required additional treatment with caspofungin and vancomycin. Sixteen days after the patient's admission to the ICU, he had septic shock with multiorgan failure. The following day an anaerobic Gram-negative rod was cultured from blood. The isolate was identified by partial 16S rDNA sequencing (MicroSeq 500 system, Perkin-Elmer, Applied Biosystems Division) as *B. fragilis*. The isolate was screened by disk diffusion for antimicrobial susceptibility, and showed very small zone diameters for metronidazole, meropenem, piperacillin-tazobactam and clindamycin. Antimicrobial susceptibility was confirmed with Etest (bioMérieux) according to the manufacturer's instructions on Brucella Blood Agar supplemented with vitamin K and haemin (Becton Dickinson). The plates were pre-reduced for 24 h at 37 °C in an anaerobic chamber before use. The isolate was resistant to metronidazole, meropenem, imipenem, clindamycin and piperacillin/tazobactam, intermediate for tetracycline, and susceptible to tigecycline. Antimicrobial susceptibility test results are presented in Table 1.

The strain was positive for β-lactamase production by the nitrocefin disk method (Cefinase; BBL, Becton Dickinson). Resistance to carbapenems was further investigated by the metallo-β-lactamase (MBL) imipenem double-ended Etest strip, which detected the presence of a functional MBL by a decrease in MIC from 48 to 1 mg l^{-1} in the presence of EDTA (Walsh *et al.*, 2002). To confirm the MBL phenotype and other mechanisms of resistance in this multidrug-resistant (MDR) strain of *B. fragilis*, the isolate was sent to the Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary, for further examination. In the isolate, with the MBL-producing phenotype, we could detect by PCR the well-known *cfiA* gene characteristic of carbapenem-resistant *B. fragilis* strains. To account for the high carbapenem MICs, we investigated its activation by insertion sequence (IS) elements, as described previously (Sóki *et al.*, 2004). In the upstream region of this *cfiA* gene, nucleotide sequencing demonstrated a novel IS*613*-like element, IS*Bf12*. The sequence was submitted to GenBank (accession no. JN801071), and its nucleotide sequence had

Abbreviations: ICU, intensive care unit; IS, insertion sequence; MBL metallo-β-lactamase; MDR, multidrug-resistance/resistant.

The GenBank/EMBL/DDBJ accession number for the sequence of the novel *B. fragilis* IS*613*-like element, IS*Bf12*, discussed in this study is JN801071.

Table 1. Cases with MDR *B. fragilis*-group infection

Breakpoint is defined by European Committee on Antimicrobial Susceptibility Testing (EUAST) guidelines, except for cefoxitin (CFX), tigecycline (TIG) and tetracycline (TET), for which the breakpoint is defined by the Clinical and Laboratory Standards Institute (CLSI). LIN, linezolid (no breakpoint available). Other abbreviations: MTZ, metronidazole; MER, meropenem; IMI, imipenem; AMC, amoxicillin/clavulanic acid; PIP/TAZ, piperacillin/tazobactam; CLI, clindamycin; LEV, levofloxacin; FLU, fluconazole; MOX, moxifloxacin; CAS, caspofungin; VAN, vancomycin; CFO, cefotaxime; CTX, ceftriaxone, CEF, cefuroxime; GEN, gentamicin; AMI, amikacin; AMP-B, liposomal amphotericin B; CHL, chloramphenicol; S, sensitive; I, intermediate; R, resistant; ND, not described; M, male; F, female; GB, UK; GR, Germany; FR, France; DK, Denmark.

Patient (age and sex)	Isolate	Clinical presentation	MIC (μg ml^{-1})										Treatment	Outcome	Country of isolation and reference
			MTZ (≤4/>4)	MER (≤2/>8)	IMI (≤2/>8)	AMC (≤4/>8)	PIP/TAZ (≤8/>16)	CFX (≤64/>64)	CLI (≤4/>4)	LIN ND	TET (≤4/>8)	TIG (≤4/>8)			
38F	*B. fragilis* (pus+blood)	Complication after elective laporatomy for vaginal-wall adhesions and tissue nodule	8 R	ND	32 R	64 R	ND	ND	ND	ND	ND	ND	CFO MTZ CLI GEN	Prolonged hospital stay and infection. Resolved	GB; Turner *et al.* (1995)
37M	*Bacteroides distasonis* (pus)	Complication after endoscopic retrograde cholangeo-pancreatography (ERCP), laparoscopic cholecystectomy and sphincterectomy. Known to have renal transplant	>32 R	>32 R	>32 R	16 R	>128 R	>128 R	>128 R	ND	ND	ND	Immunosuppressive CTX AMI MTZ MER AMP-B	Died	GB; Rotimi *et al.* (1999)
48F	*B. fragilis* (pus+blood)	Complication after ERCP for gallstones	>256 R	>256 R	>32 R	>256 R	ND	>256 R	>256 R	1.0	>256 R	ND	CEF MTZ IMI PIP/TAZ AMI CHL AMP-B LIN	Died	GB; Wareham *et al.* (2005)
71M	*B. fragilis* (blood)	Diarrhoea. Known to have Crohn's disease	0.5 S	ND	>32 R	ND	>256 R	>256 R	>256 R	ND	128 R	ND	MTZ CFO	Died	GR; Katsandri *et al.* (2006)
75M	*Bacteroides vulgatus* (pus)	Complication after surgical procedure due to gastric carcinoma	>256 R	ND	>32 R	ND	>256 R	>256 R	>256 R	ND	>256 R	ND	LEV PIP/TAZ FLU IMI	Died	GR; Katsandri *et al.* (2006)
ND	*B. fragilis* (wound)	ND	16 R	ND	>32 R	4 S	4 S	>256 R	0.125 S	ND	ND	0.1 S	ND	ND	FR; Nagy *et al.* (2011)
84M	*B. fragilis* (pus+blood)	Complication after elective laparoscopic hemicolectomy due to colon cancer	16 R	>32 R	16 R	ND	>256 R	ND	6 R	ND	8 I	1.5 S	MER MTZ CAS VAN	Died	2012 DK; this study

92 % homology to IS*613* (Kato *et al.*, 2003), 92 % homology to the IS element upstream of the *cfiA* gene of *B. fragilis* YMC00/6/496 (Roh *et al.*, 2010), and 76 % homology (not including the rightmost approximately 100 nt) to IS*614* (Kato *et al.*, 2003) and IS*614B* (Sóki *et al.*, 2004). The strain harboured 2.7, 4.2 and 7.3 kb plasmids. The *cfiA* gene could not be detected on these plasmids by Southern hybridization, so its location was chromosomal, as is usually the case for *cfiA* genes. The analysis of the metronidazole resistance mechanism was done by PCR sequencing and Southern blotting (Sóki *et al.*, 2006), and revealed a *nim* plasmid, pIP421 (Trinh *et al.*, 1995), in this strain by examination of molecular size (7.3 kb), *nim* gene type (*nimD*), the IS element activating this *nimD* gene (IS*1169*), and Southern blotting of its *Eco*RI and *Eco*RV restriction fragments. Further molecular analysis detected the *ermF*, *tetQ* and *tetX* variant resistance genes in this strain (Bartha *et al.*, 2011). *ermF* may mediate the strain's clindamycin resistance, and *tetQ* its reduced susceptibility to tetracycline; however, the strain was still susceptible to tigecycline (Bartha *et al.*, 2011).

The ICU was recommended to change antibiotics to tigecycline for optimal treatment of the patient and his infection, but because the patient was apparently improving, there was no change in treatment. On day 25 after the patient was admitted to hospital, he suffered a new event of septic shock and died.

Blood cultures taken 3 days after the first blood culture and abdominal fluid from the day before the patient died showed growth of an identical *B. fragilis* strain with the same pattern of resistance.

Meropenem was administered from day 6 after his operation to his death, and metronidazole was administered from day 6 after his operation to day 18 and then again from day 22 to his death.

Discussion

Anaerobic infections are often treated empirically, based on surveillance reports of susceptibility patterns of these pathogens (Löfmark *et al.*, 2010; Nagy, 2010; Nagy *et al.*, 2011; Seifert *et al.*, 2010). Susceptibility varies considerably among the different species in the group (Nagy, 2010; Seifert *et al.*, 2010). Routine identification to the species level and determination of antimicrobial susceptibility vary between countries and laboratories (Nagy, 2010; Nagy *et al.*, 2011), but optimal empirical therapy with appropriate antibiotics has been shown to be important for favourable clinical outcome in *B. fragilis* group bacteraemia. Insufficient treatment is associated with increased mortality rates and increased length of hospital stay (Sóki *et al.*, 2004, 2006; Nagy, 2010; Nguyen *et al.*, 2000; Dubreuil & Odou, 2010). In a Europe-wide study (Nagy *et al.*, 2011) involving 13 countries, between January 2008 and March 2009, the susceptibility of 824 *B. fragilis* group isolates against nine antibiotics was determined. Increasing resistance was observed for cefoxitin, clindamycin and moxifloxacin. The lowest levels of resistance were observed for imipenem, metronidazole (<1 %) and tigecycline. Differences were detected between geographical areas and hospitals. Similar studies have been performed in the USA (Snydman *et al.*, 2011), and they demonstrate a similar trend.

Historically, Denmark has had a low consumption of antibiotics compared with other European countries. However, this has changed during the last decade, and consumption of antibiotics is now higher than in other Scandinavian countries, according to the latest report from the European Centre for Disease Prevention and Control (ECDC; http://www.ecdc.europa.eu/en/eaad/Documents/EAAD-2011-Summary-Antimicrobial-Consumption-data.pdf).

Resistance to carbapenems is mostly related to one enzyme, the *cfiA* MBL, Ambler class B. For the expression of this gene, special IS elements are needed upstream of *cfiA* to act as a promoter (Nagy, 2010; Toprak *et al.*, 2012). However, in the study of Wybo et al., 2011, it was found that the *cfiA*-positive isolates had higher meropenem MICs than *cfiA*-negative *B. fragilis* isolates, although the *cfiA* gene was not activated. In our study the *cfiA* gene was not sequenced. Sequencing studies of *cfiA* genes show that there are molecular variants of this enzyme (García *et al.*, 2009; Toprak *et al.*, 2012), although for the high enzymic activities required for high carbapenemase production the role of the activating IS elements is crucial, as several previous studies have demonstrated (Podglajen *et al.*, 1992, 1994; Kato *et al.*, 2003; Sóki *et al.*, 2004; García *et al.*, 2009; Toprak *et al.*, 2012). The activating IS element, IS*Bf12*, in our strain was novel, but belonged to the group defined by IS*613*, IS*614* and IS*614B*. The members of this group are often mosaics of other group members, as proposed previously (Sóki *et al.*, 2004), and in our case IS*Bf12* also displayed a similar phenomenon. The whole IS, including the rightmost portion, was clearly homologous to IS*613*, but with respect to IS*614* and IS*614B*, the rightmost portion was not. The mechanisms of resistance to tetracyclines are based on ribosomal protection. The *tetQ* gene encodes a ribosomal protection protein that is responsible for most tetracycline resistance observed in *Bacteroides* strains. The *tetX* gene encodes an FAD- and NADPH-requiring oxidoreductase that inactivates tetracycline in the presence of oxygen (Bartha *et al.*, 2011). Tigecycline is a glycylcycline, a semi-synthetic derivative of minocycline, and some data suggest that in most cases tigecycline overcomes tetracycline resistance mechanisms (Bartha *et al.*, 2011), as in our case. Clindamycin resistance, in *Bacteroides* strains, is mostly mediated by a macrolide-lincomycin-streptogramin (MLS) mechanism, and the gene encoding this type of resistance is *ermF* (Nagy *et al.*, 2001).

Several metronidazole resistance mechanisms have been described (Steffens *et al.*, 2010), and are most commonly expressed by the resistance gene linked to specific nitroimidazole (*nim*) genes, named *nimA–G*, which are located on the chromosome or on a plasmid (Sóki *et al.*, 2006; Nagy, 2010; Dubreuil & Odou, 2010; Pumbwe *et al.*, 2007).

Metronidazole-resistant strains of the *B. fragilis* group have been described in several countries, but in general, resistance is low. In the study of Toprak *et al.* (2012), none of the strains showed reduced susceptibility to metronidazole or expressed the *nim* gene. As routine susceptibility testing of anaerobic bacteria in most laboratories is only performed from blood or other serious infections, it is difficult to estimate how frequent MDR *B. fragilis* group strains are from other sites of infection or in the colon. However, it has been noted in the Europe-wide study of Nagy *et al.* (2011) that 'Nonsusceptible strains to imipenem and metronidazole were more resistant to other anti-anaerobic drugs'. A few isolates exhibiting MDR to at least two antimicrobial classes, together with metronidazole and/or carbapenem resistance, have been described before in Europe (Table 1). As we do not have the full molecular profiles from these other cases, reported in Table 1, of MDR *B. fragilis* group strains, it is not possible to identify a particular gene complex or plasmid which could be responsible for or contribute to MDR in these strains.

In the study of Katsandri *et al.* (2006), it was shown in the first case described that the strain was metronidazole-susceptible but imipenem-resistant, and the authors detected a *cfiA* gene, which is associated with carbapenem resistance. There was no further description and no information about IS elements. In the second case described, the authors were not able to detect a *nim* gene, even though the MIC for metronidazole was high (>256 mg l^{-1}), indicating that there are other mechanisms, e.g. overexpression of efflux pumps, which could explain the high MIC (Löfmark *et al.*, 2010). The *cfiA* gene was also detected but not described further, and there was no information about IS elements. In the rest of the cases no molecular information was presented. As already described, resistance to metronidazole is associated with *nim* genes, and high metronidazole MICs are seen in strains with plasmid and chromosomal *nim* genes (Sóki *et al.*, 2006). Some studies have shown that *nimA* is the most prevalent of the *nim* genes (Löfmark *et al.*, 2005; Stubbs *et al.*, 2000). It has also been shown that the chromosomal *nim* genes are often associated with the presence of the *cfiA* gene (Sóki *et al.*, 2006). All in all, these findings indicate that the resistance mechanisms for MDR *B. fragilis* group strains are complex, and we still do not know to what degree transfer of resistance genes, including MDR, occurs between members of the *B. fragilis* group.

In our case, it is difficult to determine whether the patient died from, or with, his MDR *B. fragilis* strain. He had a serious underlying condition with complications, although apparently the colon cancer had been removed. However, the MDR *B. fragilis* strain was not treated sufficiently and it was possible to retrieve the strain on later occasions from blood cultures and abdominal fluid.

With this report, we would like to increase awareness of the fact that resistance is increasing in Europe against antibiotics commonly used for anaerobic bacteria, and also against carbapenems and metronidazole, while the frequent presence of the *cfiA* gene, with the potential for a dramatic increase in carbapenem resistance, is concerning. Decreased susceptibility or resistance can be responsible for clinical failures, and in cases with serious anaerobic infections it is important to improve both the bacteriological identification and the susceptibility testing of the antibiotics used for therapy. It is therefore of great importance to do routine antimicrobial susceptibility testing and not rely only on periodically published surveillance reports in cases of serious anaerobic infections.

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