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Metronidazole- and Carbapenem-Resistant *Bacteroides thetaiotaomicron* Isolated in Rochester, Minnesota, in 2014

Sapna P. Sadarangani,[a] Scott A. Cunningham,[c] Patricio R. Jeraldo,[b,d] John W. Wilson,[a] Reeti Khare,[c] Robin Patel[a,c]

Division of Infectious Diseases[a], Department of Surgery[b], and Division of Clinical Microbiology[c], Mayo Clinic, Rochester, Minnesota, USA; Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA[d]

Emerging antimicrobial resistance in members of the *Bacteroides fragilis* group is a concern in clinical medicine. Although metronidazole and carbapenem resistance have been reported in *Bacteroides thetaiotaomicron*, a member of the *B. fragilis* group, they have not, to the best of our knowledge, been reported together in the same *B. thetaiotaomicron* isolate. Herein, we report isolation of piperacillin-tazobactam-, metronidazole-, clindamycin-, ertapenem-, and meropenem-resistant *B. thetaiotaomicron* from a patient with postoperative intra-abdominal abscess and empyema. Whole-genome sequencing demonstrated the presence of *nimD* with at least a portion of IS*1169* upstream, a second putative *nim* gene, two β-lactamase genes (one of which has not been previously reported), two *tetX* genes, *tetQ*, *ermF*, two *cat* genes, and a number of efflux pumps. This report highlights emerging antimicrobial resistance in *B. thetaiotaomicron* and the importance of identification and antimicrobial susceptibility testing of selected anaerobic bacteria.

Increasing antimicrobial resistance has been reported in members of the *Bacteroides fragilis* group. Carbapenem resistance is rare and has been associated with expression of a metallo-β-lactamase encoded by *cfiA*, although other mechanisms have been reported. Metronidazole resistance is also rare, and though its mechanisms are incompletely delineated, it has been associated with plasmid-borne and chromosomal *nim* genes as well as efflux pumps (1). Some resistance genes may be present but not expressed unless activated by upstream insertion sequence (IS) elements.

In 2013, the first metronidazole-nonsusceptible *Bacteroides thetaiotaomicron* isolate was reported from Turkey; the isolate was imipenem susceptible (2). Here, we report a case of carbapenem- and metronidazole-resistant *B. thetaiotaomicron* in a patient with postoperative intra-abdominal abscess and empyema. Whole-genome sequencing was used to identify putative resistance genes.

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CASE REPORT

A 43-year-old man with a 3-year history of recurrent, medically intractable sigmoid diverticulitis underwent elective laparoscopic sigmoid resection at an outside institution. His postoperative course was complicated by abdominal pain and fevers. Computed tomography revealed intra-abdominal collections requiring placement of multiple percutaneous drains. Cultures from the intra-abdominal collections yielded polymicrobial growth of *Escherichia coli* (susceptible to meropenem, piperacillin-tazobactam, and cephalosporins, and resistant to fluoroquinolones and aminoglycosides), *B. fragilis* group (β-lactamase positive), and *Candida albicans*. To evaluate for possible colonic leakage, an exploratory laparotomy and intraoperative colonoscopy were performed on postoperative day 5. Turbid-appearing fluid was observed in the pelvis proximal to the left colon, but no frank leak was identified. The patient received intravenous (i.v.) meropenem, vancomycin, and fluconazole. Due to persistent fever and abdominal pain, repeat exploratory laparotomy and peritoneal lavage were performed with resection of the colorectal anastomosis and creation of an end colostomy. Because of continued fever and leukocytosis (19×10^9 cells/liter), the patient was transferred to the Mayo Clinic (Rochester, MN). He lived in Iowa, where he worked as a manager for a gelatin factory. He had lived in China for 2 years and had not received medical care there.

His antibiotics were initially transitioned to i.v. piperacillin-tazobactam, i.v. vancomycin, and fluconazole. All blood cultures were negative. Sinograms of the intra-abdominal drains confirmed appropriate drain placement and control of his abdominal fluid collections. A drain in a left upper quadrant intra-abdominal collection had been transpleurally placed, resulting in the development of a large left pleural effusion. Pleural fluid cultures obtained via thoracentesis grew *B. thetaiotaomicron* and *C. albicans*. The *B. thetaiotaomicron* isolate was β-lactamase positive. Susceptibility testing using gradient antibiotic strips (Etest; bioMérieux, Durham, NC) showed the isolate to be resistant (Clinical and Laboratory Standards Institute breakpoints [3]) to piperacillin-tazobactam (MIC, >256 and 4 μg/ml, respectively), metronidazole (MIC, 32 μg/ml), clindamycin (MIC, >256 μg/ml), ertapenem (MIC, ≥16 μg/ml), and meropenem (MIC, >32 μg/ml), and susceptible (U.S. Food and Drug Administration breakpoint [4]) to tigecycline (MIC, 4 μg/ml). An ultrasound-guided left pleural pigtail catheter was placed, and thrombolytic therapy was instilled to optimize pleural fluid drainage. The patient's antimi-

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Address correspondence to Robin Patel, patel.robin@mayo.edu.

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TABLE 1 Antimicrobial resistance genes identified by whole-genome sequencing in *B. thetaiotaomicron*

Resistance mechanism(s)	Function	NCBI accession no.	Nucleotide identity
ABC transporter, ATP-binding protein	Efflux pump	AE015928.1	*B. thetaiotaomicron* VPI 5482, complete genome, ABC transporter, ATP-binding protein 1814/1824 (99.5%)
RND family efflux transporter, MFP subunit (2)	Efflux pump	AE015928.1	*B. thetaiotaomicron* VPI 5482, complete genome 1081/1083 (99.8%) and 1216/1236 (98.4%)
AcrB/AcrD/AcrF family efflux transporter	Efflux pump	AE015928.1	*B. thetaiotaomicron* VPI 5482, complete genome 2975/3033 (98.1%)
Efflux pump	Efflux pump	AE015928.1	*B. thetaiotaomicron* VPI 5482, complete genome 3036/3105 (97.8%)
β-Lactamase[a]	β-Lactamase	AE015928.1	*B. thetaiotaomicron* VPI 5482, complete genome β-lactamase 871/882 (98.8%)
cat (2)	Chloramphenicol-inactivation enzyme	AE015928.1	*B. thetaiotaomicron* VPI 5482, complete genome 618/624 (99.0%) and 628/645 (97.3%)
nim-like	5-Nitroimidazole resistance	AE015928.1	*B. thetaiotaomicron* VPI 5482, complete genome 468/480 (97.5%)
ABC transporter, ATP-binding protein	Efflux pump	CP002530.1	*Bacteroides salanitronis* DSM 18170 1332/1664 (80.0%)
Putative class A β-lactamase (2 identical copies)[b]	Novel class A β-lactamase	KP233892	This report
ermF 23S rRNA methyltransferase	Clindamycin resistance	AJ311171.1	*B. thetaiotaomicron* transposon CTnDOT *ermF* region 800/801 (99.9%)
mef	Macrolide efflux	KJ816753, CP003351, CP000673.1, AY355407	*B. fragilis* strain HMW 615 transposon CTnHyb 1209/1218 (99.3%); *Enterococcus faecium* Aus0004 1198/1218 (98.4%); *Clostridium kluyveri* NBRC 12016 1178/1209 (97.4%); *Streptococcus* sp. "group G" strain 02M157741 macrolide-efflux protein (*mef*) gene 1163/1218 (95.5%)
tetX (2)	Tetracycline-inactivation enzyme	AJ311171.1	*B. thetaiotaomicron* transposon CTnDOT 1077/1077 (100%) and 1129/1167 (96.7%)
tetQ	Tetracycline resistance	CP008741, CP003274.1, AP006841.1, AY515263	*Bacteroides dorei* 1926/1926 (100%); *Alistipes finegoldii* DSM 17242 1926/1926 (100%); *B. fragilis* YCH46 DNA, complete genome 1926/1926 (100%); *B. fragilis* conjugative transposon CTn341 1926/1926 (100%)
nimD[c]	5-Nitroimidazole resistance	X76949	*B. fragilis* 491/495 (99.2%)

[a] Insertion element necessary for upregulation not detected.
[b] IS*612B* located upstream, indicating a potential role in expression.
[c] IS*1169* found upstream of *nimD* as reported in X76949, but an incomplete sequence was obtained for IS*1169*.

crobial regimen was transitioned to i.v. tigecycline, and fluconazole was continued. He improved clinically with resolution of fever and normalization of his peripheral white blood cell count. Subsequent imaging showed resolution of the fluid collections in the left upper quadrant and improvement in the left pleural effusion. The drains were removed. Because of intractable nausea and other gastrointestinal side effects, tigecycline and fluconazole were discontinued, and he was followed clinically over the next few months without disease recurrence.

MATERIALS AND METHODS

Whole-genome sequencing. The *B. thetaiotaomicron* isolate (IDRL-10114) was suspended in 1× Tris-EDTA buffer and treated with 5 mg/ml lysozyme at 37°C for 3 h. Genomic DNA was extracted using a Maxwell 16 tissue DNA purification cartridge (Promega, Madison, WI) and purified by column purification using the Genomic DNA Clean & Concentrator-10 kit (Zymo Research Corp., Irvine, CA).

Illumina next generation sequencing was performed on paired-end and Nextera mate-pair libraries using a MiSeq platform (Illumina, Inc., San Diego, CA) with a 600-cycle kit, resulting in an average coverage of 85×. Sequencing reads were processed for library adapter removal and filtering using Trimmomatic 0.32 (5) with the following parameters: ILLUMINACLIP:adapter.fasta:2:30:10 LEADING:3 TRAILING:3 MAXINFO:220:0.1 MINLEN:70. Assembly was generated with SPAdes 3.1.1 (6). Resistance genes were searched against ResFams 1.2 (7) using HMMER 3.1b2 (8). Insertion sequence elements were searched using the ISfinder tool (9).

Mayo Clinic *B. thetaiotaomicron* antibiogram. An antibiogram was generated for all *B. thetaiotaomicron* isolates subjected to antimicrobial susceptibility testing at the Mayo Clinic between November 2011 and December 2014. Susceptibility testing was performed using antibiotic gradient strips; results were interpreted using Clinical and Laboratory Standards Institute guidelines (3).

RESULTS

Whole-genome sequencing analysis. Results of the analysis of whole-genome sequence data for resistance genes using ResFams 1.2 demonstrated a number of efflux pumps, two *tetX* genes, *tetQ*, *ermF*, *mef*, and two *cat* genes (Table 1). Two β-lactamase genes were detected, one of which was 98.8% identical to a β-lactamase gene previously reported in *B. thetaiotaomicron* VPI 5482 (10) and was not associated with an upstream insertion sequence. The second β-lactamase gene was novel. IS*612B* was located upstream of it, suggesting a possible role in expression.

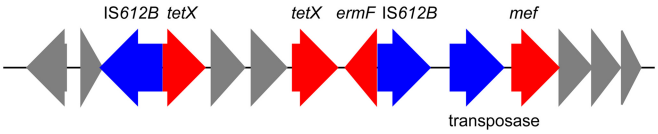

FIG 1 Arrangement of the genetic region harboring *ermF* and *tetX*. The red arrows represent resistance genes, the blue arrows are mobile/transposable elements, and the gray arrows represent other genes/open reading frames (ORFs).

ResFams 1.2 does not include *nim* genes. A separate analysis for *nim* genes showed the presence of a plasmid-borne *nimD* with at least a portion of IS*1169* (the contig terminated within the insertion sequence) inserted upstream, as previously reported in *B. fragilis* (11). There was a second *nim*-like element, which was 97.5% identical to that previously reported in *B. thetaiotaomicron* VPI 5482. *cfiA* was not detected.

Most of the detected resistance genes were highly similar to those reported in the complete genome sequence of *B. thetaiotaomicron* VPI 5482 (10). However, there were several not reported in *B. thetaiotaomicron* VPI 5482, including that for the novel putative β-lactamase, as well as an efflux pump gene, *ermF* (with IS*612B* upstream), two *tetX* genes, *tetQ*, *nimD*, and *mef*. *ermF* and one of the *tetX* genes have been previously reported on a *B. thetaiotaomicron* transposon (CTnDOT), *tetQ* has been previously reported on a *B. fragilis* conjugative transposon (CTn341), and *mef* has been previously reported on a *B. fragilis* transposon (CTnHyb). *ermF*, the two *tetX* genes, and *mef* were located in proximity to one another, possibly as part of a transposon (Fig. 1). In addition to IS*1169* and IS*612B*, IS*Bthe3* and IS*Bthe4* were detected.

Susceptibility of Mayo Clinic *B. thetaiotaomicron* isolates. From November 2011 to December 2014, 65 *B. thetaiotaomicron* isolates were tested for antimicrobial susceptibility (Table 2). With the exception of the isolate described herein, all were metronidazole susceptible; however, 17% were ertapenem nonsusceptible, 23% were piperacillin-tazobactam nonsusceptible, and 94% were clindamycin nonsusceptible.

DISCUSSION

Anaerobic bacteria, including *B. fragilis* group members, are a normal part of the human intestinal flora and can be important pathogens in intra-abdominal and postoperative surgical infections. Among anaerobic flora within intra-abdominal infections, *B. fragilis* is most commonly identified, followed by *B. thetaiotaomicron* (12). There has been increasing antimicrobial resistance reported in the *B. fragilis* group in recent years, with resistance to β-lactam antibiotics via β-lactamase production becoming progressively more common (13). Clinical *B. thetaiotaomicron* isolates from a Turkish study showed resistance to ampicillin, piperacillin, clindamycin, and on occasion chloramphenicol; however, all isolates were susceptible to metronidazole and imipenem (14). Because of emerging resistance, metronidazole, carbapenems, and β-lactam–β-lactamase inhibitor combinations, such as piperacillin-tazobactam, have been increasingly used to treat *B. fragilis* group infections. This report cautions that today, these agents should not be considered to be universally active.

The first metronidazole-nonsusceptible *B. thetaiotaomicron* isolate was from a pancreatic cancer patient in Turkey who developed *B. thetaiotaomicron* bacteremia. The isolate had a metronidazole MIC of 16 μg/ml, corresponding to intermediate based on Clinical and Laboratory Standards Institute criteria and resistant based on European Committee on Antimicrobial Susceptibility Testing criteria (2). This isolate was, however, susceptible to carbapenems and ampicillin-sulbactam. We believe that the case reported herein is the first case of concurrent metronidazole and carbapenem resistance in an isolate of *B. thetaiotaomicron* as well as the first case of fully metronidazole-resistant *B. thetaiotaomicron*.

Metronidazole resistance in the *B. fragilis* group has been associated with acquisition of *nim* genes (*nimA* to *nimH* and *nimJ*) located on the chromosome or on plasmids (15). The *B. thetaiotaomicron* isolate from the aforementioned case carried *nimE* (2). The presence of *nim* may not correlate with clinically relevant metronidazole resistance, as the gene may not be expressed or may be expressed at low levels unless activated by upstream insertion sequence elements (16, 17). The function of Nim proteins is controversial. Also, in a recent study, no correlation was found between levels of Nim and levels of metronidazole resistance (18). Whole-genome sequencing showed that our isolate had *nimD* with at least a portion of IS*1169* inserted upstream, suggesting that this may have been the mechanism underlying metronidazole resistance. A second *nim* gene similar to that reported in *B. thetaiotaomicron* VPI 5482 (10) was also present.

Carbapenem resistance in *B. fragilis* has been attributed to metallo-β-lactamase production, first described in 1986 (19). The associated gene is *cfiA*; however, not all *B. fragilis* strains carrying this gene express phenotypic carbapenem resistance (20), and carbapenem resistance has been reported in *B. fragilis* group members in the absence of *cfiA*. High-level *cfiA* β-lactamase-associated carbapenem resistance appears to be associated with the presence of an insertion element immediately upstream of *cfiA* that provides an efficient promoter (21). As mentioned, *cfiA* is not the only mechanism of carbapenem resistance in the *B. fragilis* group; Fernández-Canigia et al., for example, detected *cfiA* in only 8 out of 23 *B. fragilis* group isolates with decreased susceptibility to carbapenems, all of which were *B. fragilis* species (22). In their study, the mechanism of carbapenem resistance in *B. thetaiotaomicron* and *Bacteroides ovatus* was undefined (22). Other mechanisms of carbapenem nonsusceptibility include altered penicillin-binding proteins, efflux, and, hypothetically, outer membrane porin protein mutations (16). *cfiA* was not detected in our isolate. Snydman et al. reported three carbapenem-resistant *B. thetaiotaomicron* isolates, none of which tested positive for *cfiA* using PCR (12). Interestingly, multiple efflux pumps were detected in our isolate, suggesting a possible contribution of efflux to carbapenem nonsusceptibility. The function of the novel β-lactamase reported

TABLE 2 Antimicrobial susceptibility of 65 *B. thetaiotaomicron* isolates (Mayo Clinic, Rochester, MN), November 2011 to December 2014[a]

Antimicrobial agent	No. susceptible[b] (%)	No. intermediate[b] (%)	No. resistant[b] (%)
Ertapenem	54 (83)	1 (2)	10 (15)
Clindamycin	4 (6)	11 (17)	50 (77)
Metronidazole	64 (99)	0 (0)	1 (2)
Piperacillin-tazobactam	50 (77)	3 (5)	12 (19)

[a] Data courtesy of Nicolynn C. Cole.
[b] Clinical and Laboratory Standards Institute guidelines applied (3).

here remains unknown; the IS element IS*612*/IS*612B* located upstream of the novel putative β-lactamase has been found upstream of other β-lactamases (23).

A national survey of antibiotic susceptibility of 363 clinical *B. fragilis* group isolates collected from 2006 through 2009 from 17 centers in Argentina showed that among the 198 *B. fragilis* isolates tested, 1.5% and 2.4% were resistant to imipenem and ertapenem, respectively, and that among the 69 isolates of *B. thetaiotaomicron* and *B. ovatus* tested, 1.4% and 4.1% were resistant to imipenem and ertapenem, respectively; no metronidazole resistance was detected (22). Among 66 *Bacteroides* isolates collected between 2003 and 2008 from a Turkish hospital, 5 were resistant to meropenem, of which 4 were also resistant to imipenem; no metronidazole resistance was detected (20). Roh et al. reported four meropenem-resistant *B. fragilis* isolates recovered between 1997 and 2004 from a Korean hospital (23). A recent report from Russia describes a metronidazole- and imipenem-resistant *B. fragilis* isolate as well as an imipenem-resistant but metronidazole-susceptible *B. fragilis* strain, the latter apparently isolated from two patients, suggesting possible person-to-person transmission (24).

Limited *in vitro* data suggest high rates of susceptibility of the *B. fragilis* group to tigecycline (25). Our patient's isolate was susceptible to tigecycline, albeit with an MIC of 4 μg/ml (at the U.S. Food and Drug Administration susceptibility breakpoint), and exhibited the presence of two *tetX* genes. Although he did not tolerate a prolonged course of tigecycline, effective drainage of his intra-abdominal and pleural fluid collections resulted in a successful clinical outcome.

We and others have used whole-genome sequencing in an attempt to characterize resistance mechanisms in *B. fragilis* group members (15, 26). A recent report described an imipenem-, metronidazole-, piperacillin-tazobactam-, and clindamycin-resistant *Bacteroides* genome species strain related to *B. fragilis*; whole-genome sequencing detected several antimicrobial resistance genes in this isolate, including *cfiA13* (with an upstream insertion sequence), *cfxA*, two putative *nim* genes, *ermF*, *tetQ*, and efflux-associated genes (26). Another recent report described whole-genome sequencing of five meropenem-nonsusceptible *B. fragilis* strains, three of which were nonsusceptible to metronidazole (15). *cfiA* was detected in all five, and *nim* genes were detected in the metronidazole-nonsusceptible isolates; *tetQ*, *bexB*, *ermF*, *linAn2*, and *mefEn2* were variously detected (15). Whole-genome sequencing currently has limitations for detecting antimicrobial resistance genes in the *B. fragilis* group. Assembly of complete, finished genomes, which is ideally needed, is hindered by the frequent presence of multiple IS elements. Annotation is also problematic. A more complete understanding of the genetic basis of antimicrobial resistance in this group of organisms is needed to develop tools to use genetic data to infer susceptibility or resistance to antimicrobial agents. Sydenham et al. (15) used a combination of the ResFinder database (27) and a custom BLAST database to survey for resistance genes in their *B. fragilis* strains. Since these tools failed to detect the β-lactamases of our isolate, we instead chose to use the hidden Markov model-based ResFams (7), which, while more challenging to use, found the known β-lactamase and other resistance genes and even predicted a novel β-lactamase gene.

The emergence of antimicrobial resistance in *B. fragilis* group members raises concern for horizontal gene transfer in the human intestinal tract. The source of our patient's resistant isolate/resistance genes is unknown but might hypothetically be endogenous gene transfer/selection, travel-related transmission, or nosocomial/procedural transmission. Although theoretical, the findings presented and discussed herein raise the possibility that, as with carbapenem-resistant *Enterobacteriaceae*, resistant *B. fragilis* group species may be nosocomially transmitted (24).

Carbapenems and metronidazole were once considered to be generally active against *B. fragilis* group organisms. The Clinical and Laboratory Standards Institute guidelines provide two strategies for clinical microbiology laboratories to perform susceptibility testing on anaerobic bacteria, routine or periodic (i.e., intermittent) surveillance testing (28); the former should be the preferred strategy for clinically significant isolates of *B. thetaiotaomicron* because, as highlighted by our antibiogram data, carbapenem resistance is not uncommon in this species. Fortunately, with the advent of matrix-assisted laser desorption ionization–time of flight mass spectrometry, identification of the individual members of the *B. fragilis* group to the species level has been simplified (29), although misidentifications of individual species have been reported (26).

Metronidazole- and carbapenem-resistant *B. thetaiotaomicron* poses a therapeutic challenge for severe infections involving this organism. Our case along with other recent reports of metronidazole and carbapenem resistance in the non-*B. thetaiotaomicron B. fragilis* group (15, 24, 26) suggests that carbapenems and metronidazole, once considered to be universally active against *B. fragilis* group organisms, cannot be assumed to be active today, emphasizing the need for routine identification and antimicrobial susceptibility testing of selected anaerobic bacteria.

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