Identification and characterization of invasive multi-drug-resistant (MDR) Bacteroides genospecies in Canada

Christopher Graham¹,², Alireza Eshaghi³, Alicia Sarabia¹, Sandra Zittermann³, Patrick Stapleton³, Julianne V. Kus³,⁴ and Samir N. Patel³,⁴,*

Abstract

We identified and characterized a genome of the multi-drug-resistant Bacteroides genospecies recovered from an invasive specimen from a hospitalized patient in Canada. The strain was resistant to penicillin, piperacillin-tazobactam, meropenem, clindamycin and metronidazole. The strain harboured a plasmid containing the nimE gene, which has been shown to be associated with metronidazole resistance. The study highlights the importance of being vigilant in suspecting antimicrobial drug resistance when a patient is not improving on therapy.

BACKGROUND

Bacteroides species are one of the most abundant and important parts of the normal gut flora in humans. They are also the most significant opportunistic anaerobic pathogen. Bacteroides fragilis group, which contains multiple closely related Bacteroides species, is the most commonly encountered anaerobe associated with infections. Infections caused by the B. fragilis group include intra-abdominal infection, deep-tissue infection and sepsis. These infections can lead to severe outcomes including death if they are not treated appropriately and promptly. Resistance to several antibiotics including penicillin, clindamycin, cefoxitin and moxifloxacin has been well established but resistance to metronidazole, piperacillin-tazobactam and carbapenems remains relatively rare in North America [1, 2].

CASE PRESENTATION

A patient with no travel or hospitalization history in the last 5 years, a history of alcohol abuse, recurrent pancreatitis and a complicated ICU admission for pneumonia 7 years prior, was admitted to hospital with pneumonia. The patient was started on azithromycin and ceftriaxone. On day 3, the patient deteriorated, was moved to the ICU and treatment changed to piperacillin-tazobactam. Initially, the patient improved but on day 12, developed diarrhea; metronidazole (for possible Clostridioides difficile infection) and meropenem were added. A CT scan revealed evidence of ischaemic bowel with perforation and abscess; the patient underwent a laparotomy for source control. Meropenem was continued for 7 days but metronidazole was stopped after 2 days. During the laparotomy, an intra-abdominal swab was taken, which grew Bacteroides fragilis, herein this isolate is referred to as PHL2737. The patient remained afebrile and completed 7 days of meropenem treatment. Two days later, the patient became febrile again. A blood culture from a 17-day-old central line grew B. fragilis (peripheral culture was negative) and meropenem was restarted. The patient had ongoing fever and inotrope dependence. The central line was removed and imaging revealed an intra-abdominal fluid collection. A percutaneous drain was placed to channel purulent material, which was sent for culture and grew B. fragilis. The patient remained febrile and due to the concern of possible carbapenem resistance, linezolid was added to the meropenem without any additional interventions. The patient subsequently defervesced, inotropes were discontinued and was transferred out of the ICU on day 39 with subsequent discharge home on day 70. Bacterial isolates were sent to Public Health Ontario (PHO) Laboratory for susceptibility testing and confirmation of identification.
Table 1. Average nucleotide identity (ANI) of PHL2737 against other *Bacteroides* spp.

| *Bacteroides* strains | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|-----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1. PHL2737 (QAWD00000000, CP040630)          | 100 |     |     |     |     |     |     |     |     |
| 2. *Bacteroides* spc UW (JANI00000000.1)     | 99.97 | 100 |     |     |     |     |     |     |     |
| 3. *B. fragilis* Q1F2 (CP018937.1)          | 98.25 | 98.16 | 100 |     |     |     |     |     |     |
| 4. *B. fragilis* NCTC 9343 (NC_003228.3)   | 87.21 | 87.17 | 87.16 | 100 |     |     |     |     |     |
| 5. *B. fragilis* 638R (NC_0016776.1)       | 87.06 | 87.24 | 87.05 | 99.09 | 100 |     |     |     |     |
| 6. *B. fragilis* BOB25 (CP011073.1)        | 87.25 | 87.17 | 87.38 | 99.08 | 99.12 | 100 |     |     |     |
| 7. *B. fragilis* YCH46 (AP006841.1)       | 87.08 | 87.27 | 87.27 | 99.08 | 99.02 | 99.08 | 100 |     |     |
| 8. *B. fragilis* S14 (CP012706.1)         | 86.81 | 86.88 | 87.33 | 99.37 | 99.11 | 99.09 | 99.21 | 100 |     |
| 9. *B. fragilis* BFBE1 (LN877293.1)      | 87.17 | 87.25 | 87.36 | 99.11 | 99.16 | 99.17 | 99.17 | 99.15 | 100 |

Isolate PHL2737 was identified as *B. fragilis* using Bruker MALDI Biotyper and 16S rRNA gene sequencing at PHO Laboratory. Susceptibility results showed extended resistance to penicillin (MIC≥64 mg l−1), meropenem (MIC≥64 mg l−1), piperacillin-tazobactam (MIC≥512/4 mg l−1), clindamycin (MIC≥512 mg l−1) and metronidazole (MIC≥512 mg l−1) (CLSI M100-S28). The unusual susceptibility pattern prompted additional investigation.

Genomic DNA (gDNA) was used to prepare an Illumina library using Nextera XT (Illumina, San Diego, CA, USA). The library was sequenced on the MiSeq platform. Oxford Nanopore (Cambridge, MA, USA) libraries were also prepared following the protocol for 1D gDNA long reads using a 1D ligation sequencing kit without fragmentation and including the DNA repair step with the NEBNext FFPE DNA repair module (New England Biolabs, Ipswich, MA, USA). The library was sequenced on the MinION.

A total of 5473415 paired-end illumina reads were assembled using de-novo assembler in CLC Genomics Workbench version 8.0.1 (Qiagen, Canada); 111 contigs (range from 538 to 384650 bp; N50=141340 bp) with an average coverage of 229.5-fold were generated. Using illumina and Nanopore assemblies, hybrid de novo assembly was performed using Unicycler v0.4.8-beta to close the genome and plasmids. Annotation by NCBI Prokaryotic Genome Annotation Pipeline (PGAP) predicted 4780 CDS, including 90 RNA genes: 6 cpn60 gene sequences were 99% identical only to *B. fragilis* Q1F2, while only 89% identical to BOB25, YCH46 and 638R. The cpn60 gene sequences were 99% identical only to *B. fragilis* Q1F2, while only 89% identical to BOB25, YCH46 and 638R.

The full genome of *B. fragilis* 638R (NCBI accession no. FQ312004.1) was chosen as the reference strain to compare PHL2737. High-quality core SNVs were called using a custom pipeline [4, 5]. The meta-alignment of informative core SNV positions was used to create a maximum likelihood (ML) phylogenetic tree using MEGA 6 [5] (Fig. 1b). The ML phylogeny using core SNVs grouped the analysed sequences into two clusters; a branch containing reference 638R was separated by >25000 SNVs from PHL2737. There were only eight SNVs observed between PHL2737 and strain UW (JANI00000000.1), further demonstrating a close genetic relatedness of the two strains.

Together, these results suggest that PHL2737 is not *B. fragilis sensu strictu* as suggested by MALDI-ToF MS and 16S rRNA gene sequencing, but rather a member of a novel species within the *B. fragilis* group to which the *Bacteroides* genospecies UW strain also belongs. Interestingly, our analysis also shows that the Q1F2 strain is most similar to PHL2737 and strain UW, and thus should also be part of this novel genospecies. This data demonstrates the utility of WGS for accurate identification and characterization of challenging bacterial species. Of note, the *Bacteroides* UW strain, also MDR, was recovered from a patient who had travel and had hospitalization history in India. Our patient had not left Canada in the previous 5 years though had emigrated from India many years prior to presentation.

To identify antimicrobial-resistant genes, the WGS of PHL2737 was interrogated using ResFinder 2.1 [6]. Several
known genes involved in antimicrobial resistance were identified: \textit{nimE} (metronidazole), \textit{tetQ} (tetracycline), \textit{cfiA13} (beta-lactams) and \textit{erm(F)} (clindamycin). Additionally, a mutation in \textit{gyrA} causing a S82F substitution causing fluoroquinolone resistance was also identified.

The Nanopore sequencing revealed PHL2737 had three plasmids (8331 bp, 5595 bp and 2750 bp) none of which had corresponding complete entries in the NCBI database. It is not clear, based on publically available sequences, whether the UW strain contained plasmids or not as the \textit{nimE} gene was...
The nimE gene on the PHL2737 strain was found on one of the plasmids (Fig. 2).

Both nimE or cfiA genes have been reported in several phenotypically metronidazole-sensitive strains of Bacteroides spp. implying that their presence may not necessarily confer resistance [7]. However, the presence of a specific insertion sequence (IS) element upstream of the promoter responsible for transcribing these genes appears to be necessary for up-regulation and expression of these proteins resulting in the metronidazole-resistant phenotype [8]. Using rast, we were able to locate nimE and the associated upstream insertion sequence IS5-like element (ISBf6 family transposase) on the largest plasmid (plasmid 1) (Fig. 2). The presence of the IS upstream of nimE coupled with phenotypic resistance to metronidazole supports the role of NimE in metronidazole resistance. Similarly, the metallo-β-lactamase gene, cfiA13 and its IS1380-like element (IS613 family transposase) were found on the bacterial chromosome, suggesting this as the mechanism of β-lactam/carbapenem resistance [7].

Since 2005, reports of MDR B. fragilis from Britain, Kuwait, Greece, Hungary, Denmark and three from the USA have been reported [9–13]. Antimicrobial resistance should be considered for serious Bacteroides infections that are not responding to typical therapies such as metronidazole, piperacillin-tazobactam and carbapenems in conjunction with source control. MDR Bacteroides spp. with resistance to metronidazole and carbapenems are present in Canada and should be a consideration when treatment failures are observed with empiric therapy for Bacteroides spp. infections.

Conclusions

We identified the first MDR Bacteroides spp. causing invasive infection in Canada. Characterization of this strain using WGS data revealed that this strain is a novel Bacteroides genomospecies carrying a plasmid harbouring the nimE gene, linked to metronidazole resistance. The presence of the nimE gene on a mobile genetic element is of great concern due to the possibility of horizontal gene transfer [14]. With an increasing number of reports of MDR B. fragilis strains within the past decade, our findings further highlight the importance of ongoing surveillance to guide empiric antimicrobial therapy and to track prevalence of resistance among anaerobic bacteria.

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The PHL2737 draft sequencing reads have been deposited at DDBJ/ENA/GenBank under the accession CP040630. Subsequently the complete circularized genome obtained by hybrid Nanopore-Illumina de novo assembly have been deposited at DDBJ/ENA/GenBank under the accession numbers CP040630 and plasmid 1, CP040631; plasmid 2, CP040632 and plasmid 3, CP040633.

Author contributions

C. Graham was the treating physician, provided the clinical information, reviewed and revised the manuscript. A. Eshaghi, S. Zittermann and P. Stapleton sequenced the strain, analysed the data and reviewed the manuscript. A. Sarabia provided the isolate, oversaw initial testing and reviewed the manuscript. J.V. Kus and S.N. Patel designed the study, developed and revised the draft, and finalized the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

The case reports are exempt from ethics review and approval process as it is not considered a research. Informed consent was obtained from the patient by the treating physician.

References

1. Karlowsky JA, Walkty AJ, Adam HJ, Baxter MR, Hoban DJ et al. Prevalence of antimicrobial resistance among clinical isolates of bacteroides fragilis group in Canada in 2010-2011: CANWARD surveillance study. Antimicrob Agents Chemother 2012;56:1247–1252.
2. Snyderman DR, Jacobus NV, McDermott LA, Goldstein EJC, Harrell L et al. Trends in antimicrobial resistance among bacteroides species and Parabacteroides species in the United States from 2010-2012 with comparison to 2008-2009. Anaerobe 2017;43:21–26.
3. Federhen S, Rosseello-Mora R, Klenk H-P, Tindali BJ, Konstantinidis KT et al. Meeting report: GenBank microbial genomic taxonomy workshop (12–13 May, 2015). Stand Genomic Sci 2016;11:15.
4. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J et al. The sequence alignment/map format and SAMtools. Bioinformatics 2009;25:2078–2079.
5. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30:2725–2729.
6. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–2644.

7. Trinh S, Haggoud A, Reyssset G, Sebald M. Plasmids pIP419 and pIP421 from Bacteroides: 5-nitroimidazole resistance genes and their upstream insertion sequence elements. Microbiology 1995;141 (Pt 4):927–935.

8. Gal M, Brazier JS. Metronidazole resistance in Bacteroides spp. carrying nim genes and the selection of slow-growing metronidazole-resistant mutants. J Antimicrob Chemother 2004;54:109–116.

9. Urbán E, Horváth Z, Sóki J, Lázár G. First Hungarian case of an infection caused by multidrug-resistant Bacteroides fragilis strain. Anaerobe 2015;31:55–58.

10. Merchan C, Parajuli S, Siegfried J, Scipione MR, Dubrovskaia Y et al. Multidrug-Resistant bacteroides fragilis bacteremia in a US resident: an emerging challenge. Case Rep Infect Dis 2016:2016:3607125.

11. Kalapila A, Pergam S, Pottinge P, Butler-Wu S, Whimbey E. Multidrug-Resistant Bacteroides Fragilis, 62. Seattle, washington: MMWR; 2013. p. 694.

12. Sherwood JE, Fraser S, Citron DM, Wexler H, Blakely G et al. Multi-Drug resistant Bacteroides fragilis recovered from blood and severe leg wounds caused by an improvised explosive device (IED) in Afghanistan. Anaerobe 2011;17:152–155.

13. Ank N, Sydenham TV, Iversen LH, Justesen US, Wang M. Characterisation of a multidrug-resistant Bacteroides fragilis isolate recovered from blood of a patient in Denmark using whole-genome sequencing. Int J Antimicrob Agents 2015;46:117–120.

14. Sóki J, Hedberg M, Patrick S, Bálint B, Herczeg R et al. Emergence and evolution of an international cluster of MDR Bacteroides fragilis isolates. J Antimicrob Chemother 2016:71:2441–2448.

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