

Characterization of a Multidrug-Resistant, Novel *Bacteroides* Genomospecies

Technical Appendix

Technical Appendix Table 1. ANIb values for the clinical isolate and other *Bacteroides* species*

Species	<i>B. fragilis</i> strain			Clinical isolate	<i>B. helcogenes</i> P 36–108	<i>B. thetaiotaomicron</i> VPI-5482	<i>B. xylanisolvens</i> XB1A	<i>B. vulgatus</i> ATCC 8482	<i>B. salanitronis</i> DSM 18170	<i>Parabacteroides distasonis</i> ATCC 8503
	638R	YCH46	NCTC 9343							
<i>B. fragilis</i> 638R	100	98.58†	98.56	86.63	72.57	75.28	75.3	71.1	69.49	67.43
<i>B. fragilis</i> YCH46	98.76†	100	98.6	86.64	72.52	74.42	74.97	70.89	70.54	67.47
<i>B. fragilis</i> NCTC 9343	98.77	98.59	100	86.64	72.72	74.28	74.22	70.82	69.53	67.36
Clinical Isolate	86.54	86.43	86.28	100	72.39	75.16	75.63	72.03	70.63	68.02
<i>B. helcogenes</i> P 36–108	72.65	72.67	72.86	72.69	100	72.7	72.67	70.76	69.98	67.43
<i>B. thetaiotaomicron</i> VPI-5482	74.9	74.04	73.97	74.93	72.52	100	80.42	71.19	69.5	68.14
<i>B. xylanisolvens</i> XB1A	74.9	74.63	74.11	75.1	72.4	80.7	100	71.25	69.91	67.4
<i>B. vulgatus</i> ATCC 8482	71.01	71.06	70.86	73	71.05	72.06	71.49	100	71.18	68.62
<i>B. salanitronis</i> DSM 18170	69.75	70.66	69.73	70.81	70.16	69.99	70.16	70.97	100	68.07
<i>Parabacteroides distasonis</i> ATCC 8503	67.72	67.45	67.74	68.05	67.41	68.53	67.41	68.32	67.77	100

*Bold font indicates ANIb values >95%, ANIb, average nucleotide identity by BLAST.

†Pairwise ANIb values demonstrate minor differences due to inherent stochasticity in the calculation (1).

Technical appendix Table 2. Antibiotic resistance genes in clinical isolate

Resistance_type	contig_id	feature_id	start	stop	strand	RAST-assigned function	figfam_identification	nucleotide_sequence	aa_sequence
putative nitroimidazole resistance	427	fig 6666666.52503.peg.184	47597	48070	+	Pyridoxamine 5'-phosphate oxidase	FIG01045148	atgaaacagtatactgaaacaaaagacagtagaagaattatccccctgcatattgtcttctgctgcaacacgctcaggacacccattatgtatccctca gaacctcgcatcaaggagggatgatcattctggcccaacgagcagcagatgtagctggcaaaacaacccgtgacatccctcagtagaaccc gaattgtattcccaccccagggttatcattctgagtagcagcaaaaggtaggttgcagagagacgctgaactttcgaacacctgaagaaaaactgaa ggctcaatattatggccattatgacgcgggaatttatactcatgatccgcagtagaagaatgaaaactcggagatcagattgaaacgttagccaaaa gagtagcggctgccaccatcaaaa	MKTVIENKEQVEIISRDCIFVGLTDLNRPYPVPMNFVYQDDGVY LHSGPTSSDIOMLAXNNRVICTVSUDELHFDQHPKVCASVYMRMRAKS VICGRFNFIDLEEKREALNLRHYSREFVSDPAVRRVWKIWEIP IENVTAKEYVAPHK
putative nitroimidazole resistance	500	fig 6666666.52503.peg.3895	62001	61498	-	Pyridoxamine 5'-phosphate oxidase	FIG01045148	atggaatatacaacgattaaatcgcaggaagaccctttatcgaagacagcaagcaatgatttactgaaaaggagaatcaggagtgtatctatttccacctga agaagggtttatggcattcccattaatatgtatgggataaacaacagctctctcttctattcttctgtcccaagggcgttaagttgtatactcaagactgaaatcaggca tcgttttcggttaggagcgcgcgctgtttccatcaatttagcagatagaagatgatcgtataacggactgatgttcagttagaagaactgagaagc aaaggcattgaaactctgctgataaatcttccggaagaataaaagggggttgaagatattggaagaagcttttcacgtaccacgctgtaaacgttatataaaa caatatccggaagtgtaagaagaaatgaatccctcctcggatataa	MEYINDLRKDRLLSEDEAMSLNENGVLSISPEEGYVPIY VWDDQSQSYHFACEGRKLCLQDCNQASFCVGRTRVVSQFST EYESIVINGMICOLEETEKRALLELLDKYSPEDKGLVLYMEKFSH RTHVLKLVKITSIGCKMNGPCPDY
cfiA14 Beta-lactamase	439	fig 6666666.52503.peg.820	32936	32187	-	Beta-lactamase	FIG00021206	atgaaaacagatattatccctatccctccttctcctgctgagctatggcacaagcaaaagcgtaaaaatccgatgatcacccaactcggpacaagatgta acttctattccctgcgaaatcgaagatggggatggcttccctcaacgggtaggtttatcaacaacacacccagcagcgtgtcggpacaacacatgaatgaacac aaacggaaatactggtaactggggtagcctttgtagtcccaagctaccagttttccgacccctggc-acgggtaggttatggcagctgggttcctcgaag gaagaagcttcaatcatcagcaacagatgagtagactcctcaagaagaagggttgccagcaagaacagattgtaacgcttcgagcttcaacggctggag gcatcctcccaatctattttaggaagggagcagcaccacacatcatgtattggctgacagcagagatatacctttttggagatgtatgaagaacacccag ggcacaacatctcgaatctcagagcgacagcatgagccagaaactcagataggtaaaagcaagctaccctgctgctgctgctgctgctgctgctgctg cgataggggaacccaatgatagacataccaacagagatgtaaacataatagaagaacacctcaaacatag	MKTVFIIISMFLPVAMQKSVKISDDISITLSDKVVYVYLAIEG WGMVPSNGMIVMNHQALLDPNDACAELVVMVYDLSLHAKV FTPIIMHHKDCIGGLGLYLRGGSVSXANQTDLAKELGVPE HGFDLSLVLDGMLQCYVLGGWATDNVVLWPTENLPGCM LKNQATSIGNISDQAVTWPKTLKVKKAFSPRVVPGHGDYG GLTEIHLQXVWQYIESTSKP
TetQ Tetracycline resistance protein	461	fig 6666666.52503.peg.1666	15624	13699	-	Tetracycline resistance protein T	FIG01956083	atgaataataaattaggaaactctgctacattgatgcagaaaaaactcctaaccagagaactcgtggtccagtggaacagcaaaagtgccgctgagata atggtaacacaaacagactcattgatagagaagaacagtagagaattactcggcttccatcattattatcggaaatggagtgaatcaatcattgacact ccgggacacatgattttgcgaagtggaacgacattcaaaacttgtaggagcagctctcatcttcgcaagaagacgacacgaacagcagaagtctg ttcagcttaacaaaactcccaactataatattatcaatgaatgacgctcgggtgaaattggagcgtgttgatgatataaaaaaacatctgcccaca gatctcctgtttcaaacctgttgcatgatcgttttccgttgcctccaaatataaagaagaatcaagaattgtatcacaacgtgagcagcagatata gaacgatattggcagtagaataatccgctgattgaaatcagataactcctgtgccaagaacaaagctcctcggctcagatgacagcagatgcaac tatcgatatacagagtgtggaagcattctctttatctcctgacccagctcaaacagctttcagcttctcataagatagacatgacccaagggcat aaagaagtttctaataatggcagaactcgaactcgaagctgaaagcattgaaagacacagctgcaaaaatcacaagataaaaatcaagaacttatcagg cagagagataaaatgtagaagggggcaatgatacagcagtagaagaatagaagatttccagctgagatattaggctcaaacctgtttgattcaagga ttatctcagcagcctcctcaactccctgcccacaataagccgagagagaagaacagatgcatcctgcaatcattgattgagatgaagaccctgttct ctttccataaacatagtagaattggaactgttatatgttggccaagaagatacagacattcgtgaagaacagcttccgaaggctcattgga tgagatcaagactctcacaagaacacctataaaagctcaatgaattcagctgaagtagcaccacaacccaactcctgcgccaataggctgactctgaacc cttaccctggagccagggtgcaaacgaagtagcactcctgattattcgaacattcttccaaaaggccgtttgaaaggatgtagtctgcaactcgtttac atgattggaagtagcagatcgaagaacttcaacaacgagattatagccggttaagtagcagtagacacgctgattcagaagcagctaccctctgctcaggctgctt tgcaacagcaggtggagcattcgaacccagctcctgttgatgagcagatcccaacgtagcagctcgaagcttatcagattgcaaaaaactgtagctgagatt gaagacactcagttgataagtaggtgctcattaaaggaaagttcattaaatacagaagtagactctcagaagtaggtctgacataaggctaggctaggcattt tatgttagcctagggtatacaatacaaaagagatttctgataatcagcagtagaagaagaagaaactttatcattgctcaaaaatcaagctcattaaat aa	MMINILGILAHIDAGKTSVTENLFLSAGATEKCRVNDGDTDSM DIEKRRIGTAVRSTSIWVGKNIIDTDPHGMDFIERTFKMLD GAVILSAKEGIQAKTLFSLKQLQIPIIFINKIDRAGVNLERYM DIXNLSQVDLFMTQTVDSGYPVCSQTYKEEYFVCHNDDLD ERYLADESIPADVWLYLKHIAKAPYPLVHSGAMFNIGNELDAI SSFILPPASVSNRLSALYKHIEHPKRSFLKIDGSLNRDVRNR DSEFKIKLNKTYQGREINVEGVANDIAVEDIEDIRFDYLGAKP CLIQGLSHQH PALKSSVRPNKPEERSVIALNLTWIEDPISLFSNSY SDELEISLVLQKEIQTLEERFVYHFKRPIKVVNIQI EVPMPYWAFTGLTLEPLGAGIQESDYSYHNSFCQNAVFEGR MSCQSGLHWELVDLFTFAEYSPYSPADRFQLTPYFRLL QCSQVDIIEPLMCFELQIPIQVASKATDLKLMSEIISCHSNWIC HMKKVLNLSKDYSEVSSYTKGLGFMVPCQYKITDGVSDNIR MNEKDKLFLMFQKSMSLK
cfxA Beta-lactamase	487	fig 6666666.52503.peg.3116	79479	80444	+	Beta-lactamase (EC 3.5.2.6)	FIG00000747	atgaaaaaagtgaagaaacccctgtaatttggtagatcttagttatcatagtttttatttattctcctaaactccttaataatgataaactccct ttgacagatgtttagtagacatttccgtagatcagctctgctgtgaaatggatggcaattataatacaacagacagattgaagtatgataagatt gatactctatgtagtttaaaagttctcagccattagctcttcaatgatttgaacaaagggatctactgatacttgaagaagatgagaagatgactg attcaagactggagctcattgaaagaattatccaggtttgattatcattcaaccgtagagatttctgcttactatctgaagtgaaatgaaatgaagaact gatgtttaaagaatgtagctttctcaacagcagctttagagccacctctcctctcaggtttcagatgctatcaagaagaatggctgtagcagcaga tagggcttcttataatcacttctctgttgcagcttgtagaacctgtttacagaagatattgtagtagaagaagtagttataagaatacattaa gaatgtagcaagtagcagtagatgtagctcctcgtcttgaagaagaaggtttctatgacatagcaagcgttctgtagtcaatgaaatgattattcagca tcaacaaatgtagctatagcctcctcaatagtctgacttactagatatttgaagtttaaggttaaggttaaggaatcaacagcacaatgattgtagcatala tcagaggtgtagatttcttatacaaaattcggcaactcttaa	MKKSRRKLVLQALQIVLVFLKSSVSNSTNPPDLVLTDSIRI VSAQGEIGVAIINNTDITVNNDSIVPMMSVFVHGLALCNDF DNKGSLDTLVIDRNLDSKTSWPMKDYSELVLTDRIDLRTI AQSDNINSAMFKDMVNAVQDTSFIATLPIRSSQVIAEEMSA DHRDAFYNYSVLAAMLMNRLFTSIVYGEKFSRNKLKCYTG TDRIVAPLDRKSIHAGLTSVNSGVENIGLAAVNDVIRVLPNVN CYTLAIFVDKRXGNSQSQSVVAHVHSEVYVSLIIONAIP
erm(F) macrolide resistance	442	fig 6666666.52503.peg.976	28325	27525	-	Dimethyladenosine transferase (E	FIG00000225	atgcaaaaaaagaatcccgctgttctcggctcagccacttactatgataagctgaataaaagatgcaataagacacaataatgaatcaggatagcttt tagatattgggcagcagggtttcttacttcttataaaaaactcgaacaatgtagttagttgaaacacagcagcttggtagaacattcgaaaaatttct tggtagcgcgaagttgaaatggtcaggtttgagaaatggctcagcgaattcttcaagtggtcgaatctcttggatcgaattcctgacattcctcgaat cctgattttggagccttagaattttggaaggttcatgtcttgtaagaactcctgtagaacctccacaagaagattttcagagaagcttcaactctatacgt ctttttttgtaaactgtataggttagctcgaaggtttctccaccgcaactgcaaacctcctgtaaaacttaaagaacaggttaattttttttgaaat ttaaagcaaaactatttcttctgtcttagaagaactcattatcgaaaacagctttaaagctgatttccagaagaactgagctcaggttcaatctggaaa aattcggttaaaccttagtcaaaattgtttgttcgaagcattggtagctttgttggaaatggaagtcttcccaaaaattctatctctgtag	MTKXKLPVRFQGHFTDKVLKIDARIQANISNDTLDIGAGKFL TYVILKIANNVNDALVELHRLFSDARRVQVWCFDRNFAY PKFPPKAVSNIPYDTSFIDMLFESLGNFLKSDVSNLQLEPTKLSFR KLVNYTYFYHTFOLKLVYEFSEFPPYKLSALLNKRQLQDFD KFAKYLALFTEKFLDKLVKLSKFSRQRVRSKFLNNAQV CLSPSQWVNFLEWVPEKFKHPS

Table 3.

AniB Values for additional multi-drug resistant isolates

	<i>Bacteroides fragilis</i> 638R	<i>Bacteroides fragilis</i> YCH46	<i>Bacteroides fragilis</i> NCTC 9343	<i>Bacteroides fragilis</i> HMW 615	Clinical Isolate	<i>Bacteroides fragilis</i> HMW 610	<i>Bacteroides fragilis</i> HMW 616
<i>Bacteroides fragilis</i> 638R	100*	98.56	98.58	98.3	86.63	86.58	87.23
<i>Bacteroides fragilis</i> YCH46		100	98.59	98.61	86.64	86.59	87.25
<i>Bacteroides fragilis</i> NCTC 9343			100	98.18	86.64	86.79	86.93
<i>Bacteroides fragilis</i> HMW 615				100	86.17	86.55	87.2
Clinical Isolate					100	98	96.66
<i>Bacteroides fragilis</i> HMW 610						100	97.56
<i>Bacteroides fragilis</i> HMW 616							100

*bold indicates ANiB value above 95%

Susceptibility Testing

A 0.5 McFarland suspension of organism was made in sterile saline and immediately inoculated to Brucella agar with 5% sheep blood, hemin and vitamin K (Remel, Lenexa, KS, USA). The *B. fragilis* ATCC 25285 strain was used for quality control purposes. E-tests corresponding to the antimicrobial drugs shown in the text Table were used in accordance with the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France). All plates were incubated under anaerobic conditions for 42–48 hours before reading. Where available, antimicrobial susceptibility test results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) MIC interpretive standards for anaerobes. Resistance to metronidazole and imipenem was confirmed by macrobroth dilution using Brucella broth supplemented with hemin (5 µg/mL), vitamin K1 (1 µg/mL) and lyse horse blood (5% v/v). Doubling dilutions of metronidazole and imipenem over a final concentration range of 0.05–64 µg/mL were tested and the tubes incubated anaerobically for 48 hours before reading.

Reference

1. Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A. 2009;106:19126–31. [PubMed](https://pubmed.ncbi.nlm.nih.gov/19126/)
<http://dx.doi.org/10.1073/pnas.0906412106>