



Clinical Outcomes and Molecular Characteristics of *Bacteroides fragilis* Infections

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Bacteroides fragilis is the most common opportunistic anaerobic pathogen. In the absence of appropriate antimicrobial therapy, mortality rates associated with *B. fragilis* group infections can reach as high as 50%. Therefore, we aimed to elucidate the clinical characteristics and outcomes of *B. fragilis* infections and the molecular genetic characteristics of *B. fragilis* isolates. Forty *B. fragilis* clinical isolates were collected at Hanyang University Hospital between January 2022 and December 2023. Antimicrobial susceptibility was tested using the agar dilution method. Whole-genome sequencing was conducted using the Illumina platform (Illumina, San Diego, CA, USA). Various multilocus sequence types of *B. fragilis* were identified, including ST149 (N=4), ST11 (N=4), ST1 (N=3), ST21 (N=2), and ST157 (N=1). The insertion sequence (IS) IS1187, located upstream of *cfiA*, was associated with high-level carbapenem resistance in the ST157 isolate. *B. fragilis* toxin genes (*bft*) were identified in 30% of isolates. The most common comorbidities were diabetes mellitus (26.5%) and non-metastatic cancer (23.5%). Five patients (14.7%) died within 30 days, and two (5.9%) deaths were directly attributable to *B. fragilis* infection. The emergence of high-level MIC carbapenem-resistant *B. fragilis* ST157 has led to caution in the presence of *B. fragilis* infections.

Key Words: Bacteremia, *Bacteroides fragilis*, *cfiA*, Intraabdominal infection, ST157

Bacteroides fragilis group (BFG) are residents of the healthy intestinal microbiota, but are frequently isolated from anaerobic infections [1]. In the absence of appropriate antimicrobial therapy, mortality rates associated with BFG infections can reach as high as 50% [2]. Antimicrobial resistance (AMR) profiles vary across BFG members [3, 4]. Among the BFG, *B. fragilis sensu stricto* (BFSS) is the most prevalent anaerobic microorganism in clinical specimens. Genetic divergence at the species level has been observed between division I and division II BFSS bacteria [5]. Division I BFSS harbor the cephalosporinase gene *cepA* and generally are susceptible to frontline β -lactam regimens. Division II BFSS can achieve high-level carbapenem resistance through insertion sequence (IS)-mediated activation of the chro-

mosomal carbapenemase gene *cfiA*, which is absent in division I BFSS [1, 6]. Numerous virulence factors contribute to the pathogenic potential of BFSS, including adhesin and capsule synthesis, relative oxygen tolerance, and antigenic variation in surface structures [7]. *In-vitro* studies have indicated that *B. fragilis* toxin (*bft*) may induce intestinal colonic pathology, including inflammation and carcinogenesis [1, 8]. We aimed to elucidate the clinical characteristics and outcomes of BFSS infections and the molecular genetic characteristics of BFSS isolates for the first time in Korea.

Bacterial isolates were consecutively collected at Hanyang University Hospital (Seoul, Korea) between January 2022 and December 2023. In total, 40 BFSS isolates were obtained from

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40 patients. The most common specimen was blood (N=25, 62.5%), followed by pus (N=10), tissue (N=4), and pleural fluid (N=1). Demographic data included age and sex, and underlying comorbidities were recorded using the Charlson comorbidity index [9]. Additional medical conditions noted included the use of chemotherapeutic and immunosuppressive agents, bedridden status, history of previous admissions, and anaerobic antimicrobial use prior to admission. Clinical features associated with severity included the presence of hypotension and the use of inotropics. Laboratory findings at the time of bacterial culture included white blood cell count and C-reactive protein, procalcitonin, blood urea nitrogen, and creatinine levels. Therapeutic interventions included the use of anaerobic antimicrobials within 48 hrs of bacterial culture and any surgical intervention to control the infection source. Clinical outcomes were assessed based on 30-day mortality. To evaluate clinical severity, we calculated Pitt's bacteremia score [10]. Anaerobic antibiotics included carbapenems, cefotetan, ampicillin-sulbactam, amoxicillin-clavulanate, piperacillin-tazobactam, cefoperazone-tazobactam, clindamycin, moxifloxacin, and metronidazole. The type of infection and infection-related mortality were determined by the attending physicians and confirmed by an infectious disease specialist through a medical record review using the CDC/NHSN surveillance definition [11]. The Institutional Review Board of Hanyang University Hospital approved the study protocol (IRB No. 2022-07-037). Informed consent was waived for this study.

Bacterial species were identified using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MS) system (Bruker Biotyper MS; Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility was tested using the agar dilution method according to CLSI guidelines [12, 13]. The culture medium was Brucella agar supplemented with hemin and vitamin K1 (Sigma-Aldrich, Seoul, Korea) and 5% laked sheep blood. The antimicrobial powders used included penicillin, piperacillin (Sigma-Aldrich), tazobactam (Yuhan, Seoul, Korea), cefoxitin (Merck Sharp & Dohme, West Point, PA, USA), cefotetan (Daiichi Pharmaceutical, Tokyo, Japan), clindamycin, imipenem, meropenem, moxifloxacin and metronidazole (Sigma-Aldrich). An inoculum of 10^5 colony-forming units was streaked onto a culture plate using a Steers replicator (CMI-Promex, Pedricktown, NJ, USA), and the plates were incubated in an anaerobic chamber (Bactron; Sheldon Manufacturing, Cornelius, OR, USA) at 35°C for 48 hrs. The minimum inhibitory concentration (MIC) was determined as the concentration at which a marked reduction in growth occurred, such as the transition from confluent colonies to a haze, <10 tiny colonies, or several normal-sized colonies

[12]. *B. fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were used as quality control strains. Bacterial isolates cultured on Brucella blood agar plates were sent to Macrogen (Seoul, Korea) for whole-genome sequencing (WGS) using the Illumina platform (Illumina, San Diego, CA, USA). *De-novo* assembly and validation were performed for all 40 isolates. The WGS data were analyzed using Center for Genomic Epidemiology resources (<http://www.genomicepidemiology.org/>) and PubMLST (<https://pubmlst.org/>). Multilocus sequence types (MLSTs), AMR gene repertoires, and toxin genes were determined. The IS element was identified using ISfinder (<https://www-is.biotoul.fr/index.php>).

Six cases (15.0%) were classified as colonization rather than infection. In all colonization cases, the bacteria were isolated from a wound site near the anus. The clinical characteristics and outcomes of patients with *B. fragilis* infection (N=34) are provided in Table 1. The mean age was 65.5 ± 18.7 yrs, and 32.5% of patients were female. The mean Charlson comorbidity index was 2.6 ± 2.8 , and the most common comorbidities were diabetes mellitus (26.5%) and non-metastatic cancer (23.5%). Five patients (14.7%) were bedridden, 12 (35.3%) had a history of admission within the past 3 months, and 10 (29.4%) used anaerobic antimicrobials within 1 yr prior to admission. The most common type of infection was intra-abdominal infection (58.8%), followed by skin and soft-tissue infection (14.7%) and empyema (8.8%). Among patients with intra-abdominal infection, 90.0% (18/20) exhibited bacteremia caused by *B. fragilis*, and 25.0% (5/20) had a history of major gastrointestinal tract surgery.

Polymicrobial pathogens in the same specimen were identified in 23 patients (57.5%). Low blood pressure was measured in 24.2% (8/33) of patients and 12.1% (4/33) of patients who used inotropics at the time of culture. The mean white blood cell count and C-reactive protein level were $16.4 \times 10^9 \pm 7.1 \times 10^9$ /L and 221 ± 114 mg/L, respectively. More than 80% of patients (82.4%) received anaerobic antimicrobials within 48 hrs after culture, and seven patients (20.6%) underwent surgery to treat *B. fragilis* infection. Five patients (14.7%) died within 30 days after culture, and two deaths (5.9%) were directly attributable to *B. fragilis* infection.

The resistance rates in our study were comparable to those reported in previous studies in Korea (Table 2) [3, 14]. All isolates were penicillin-resistant. The resistance rate to cefotetan (12.5%) was higher than that to cefoxitin (5.0%). The resistance rates for clindamycin and moxifloxacin were 37.5% and 15.0%, respectively. Piperacillin-tazobactam, imipenem, and meropenem were active, with 2.5% resistance rates. None of the iso-

Table 1. Clinical characteristics of 34 patients with *Bacteroides fragilis* infection

Characteristic	Value
Age, yrs	65.5 ± 18.7
Female sex	13 (32.5)
Underlying comorbidities	
Diabetes mellitus	9 (26.5)
Non-metastatic cancer	8 (23.5)
Chronic liver disease	6 (17.6)
Chronic renal disease	5 (14.7)
Metastatic solid tumor	4 (11.8)
Dementia	3 (8.8)
Cerebrovascular accident	2 (5.9)
Chronic pulmonary disease	2 (5.9)
Myocardial infarction	2 (5.9)
Congestive heart failure	2 (5.9)
Peripheral vascular disease	1 (2.9)
Hemiplegia	1 (2.9)
Other medical conditions	
Chemotherapeutic agents	1 (2.9)
Immunosuppressants	3 (8.8)
Bedridden status	5 (14.7)
History of admission within the past 3 months	12 (35.3)
History of admission within the past 1 yr	18 (52.9)
History of anaerobic antibiotic use within the past 3 months	5 (14.7)
History of anaerobic antibiotic use within the past 1 yr	10 (29.4)
Hypotension	8/33 (24.2)
Inotropics	4/33 (12.1)
Type of infection	
Intra-abdominal	20 (58.8)
Skin and soft tissue	5 (14.7)
Empyema	3 (8.8)
Others	6* (17.6)
Clinical severity	
Charlson comorbidity index	2.6 ± 2.8
Pitt's bacteremia score	0.9 ± 1.4
Therapeutic interventions	
Use of anaerobic coverage antimicrobials within 48 hrs after culture	28 (82.4)
Surgical intervention	7 (20.6)
Outcomes	
30-day mortality, all-cause	5 (14.7)
30-day mortality, infection-related	2 (5.9)

Table 1. Continued

Characteristic	Value
Laboratory findings	
White blood cells ($\times 10^9/L$)	16.4 ± 7.1
C-reactive protein (mg/L)	221 ± 114
Blood urea nitrogen (mmol/L)	14.75 ± 14.78
Creatinine ($\mu\text{mol/L}$)	194.52 ± 254.42

Continuous variables are presented as mean ± standard deviation.

Categorical variables are presented as N (%).

*These included complicated urinary tract infection (N = 2), pyogenic arthritis (N = 1), osteomyelitis (N = 1), acute cholangitis (N = 1), and primary bacteremia (N = 1).

lates were resistant to metronidazole. Among β -lactam resistance genes in BFG, *cepA*, *cfxA*, and *cfiA* are associated with resistance to penicillin, cephamycin, and carbapenem, respectively. In our study, 38 isolates (95%) were division I BFSS carrying *cepA* and showed penicillin resistance. *cfxA* was detected in 13 isolates (32.5%), among which nine were non-susceptible to cefotetan. Two isolates (5%) (Nos. 20 and 25) were division II BFSS carrying *cfiA*. The Bruker Biotyper MS system identified these as *cfiA*-positive *B. fragilis*, as previously reported [5, 15]. Isolate No. 20 exhibited high-level resistance to both imipenem (MIC, 64 $\mu\text{g/mL}$) and meropenem (MIC, 128 $\mu\text{g/mL}$), whereas isolate No. 25 was susceptible to imipenem (MIC, 4 $\mu\text{g/mL}$) and meropenem (MIC, 8 $\mu\text{g/mL}$). The presence of the *cfiA* gene does not necessarily imply resistance, as imipenem MICs in division II BFSS ranged from 0.06 to > 32 mg/L in a previous study [15]. Silent chromosomal *cfiA* can be overexpressed upon insertion of a single mobile genetic element, leading to phenotypic carbapenem resistance. The conserved ISs IS1186, IS1187, and IS613 located upstream of *cfiA* have been associated with high-level carbapenem resistance [6, 16]. In our study, IS1187 was detected upstream of *cfiA* in isolate No. 20 but not in isolate No. 25.

Nielsen, et al. [17] constructed an MLST scheme for *B. fragilis* in 2023. They found that isolates with the same ST always belonged to the same division. All ST157 isolates belonged to the division II BFSS cluster CC7 and originated from Asia. ST119 and ST140 were dominant in North America and Europe, respectively. In our study, WGS analysis revealed highly diverse MLST patterns among *B. fragilis* clinical isolates (Table 2). The 40 isolates were distributed into 31 unique STs, including ST149 (N = 4), ST11 (N = 4), ST1 (N = 3), ST21 (N = 2), and ST157 (N = 1). ST157 and ST122 were associated with division II BFSS. Notably, the ST157 isolate was highly resistant to carbapenem. The

Table 2. Antimicrobial susceptibility patterns and molecular characteristics of *Bacteroides fragilis* clinical isolates

Strain	Specimen	ST	MIC (mg/L)									β-lactam resistance genes			Toxin gene
			PEN	FOX	CTT	PIP/TAZ	IMP	MEM	CLN	MOX	MET	cepA	cfxA	cfiA	bft
1	Tissue	69	32	4	4	0.25	0.25	0.12	0.5	0.06	1	+	-	-	-
2	Pus	105	64	2	4	0.25	0.25	0.12	1	0.5	1	+	+	-	-
3*	Pus	1	256	8	32	1	0.5	0.5	1	8	0.5	+	+	-	bft-2
4	Blood	27	16	4	4	0.25	0.25	0.12	128	0.06	0.5	+	-	-	bft-1
5	Blood	21	256	8	16	1	1	0.5	0.5	8	0.5	+	+	-	-
6	Blood	17	16	4	4	0.25	0.25	0.12	256	0.06	1	+	-	-	-
7	Blood	106	32	4	4	0.25	0.25	0.12	0.5	0.12	1	+	-	-	-
8	Pus	56	256	4	8	0.5	0.25	0.25	256	16	0.5	+	-	-	bft-2
9*	Blood	9	8	4	4	0.25	0.25	0.12	2	0.06	1	+	-	-	-
10*,†	Blood	1	16	4	4	0.12	0.12	0.12	2	0.06	0.5	+	-	-	bft-2
11	Pus	4	32	4	4	0.25	0.25	0.12	0.06	0.12	1	+	-	-	-
12†	Blood	10	32	8	4	0.5	0.25	0.12	0.5	0.12	1	+	-	-	-
13	Pus	1	32	8	4	0.25	0.12	0.12	1	0.5	1	+	-	-	bft-1
14	Pus	21	256	16	64	0.25	0.25	0.25	256	0.12	1	+	+	-	-
15	Blood	39	256	8	8	0.25	0.5	0.25	256	0.06	0.5	+	-	-	bft-3
16*	Blood	48	16	4	8	0.25	0.25	0.12	0.5	4	1	+	-	-	-
17	Pus	149	16	4	4	0.12	0.25	0.12	256	0.06	0.25	+	-	-	bft-3
18*	Blood	146	128	8	32	0.06	0.25	0.12	256	1	1	+	+	-	-
19	Pleural fluid	11	16	8	8	0.12	0.25	0.06	4	0.06	1	+	-	-	-
20	Blood	157	256	64	64	256	64	128	256	0.06	2	-	-	+, IS1187	-
21	Blood	7	32	4	4	0.25	0.12	0.12	0.5	0.06	0.5	+	-	-	-
22	Blood	98	16	4	4	0.25	0.12	0.12	0.5	0.06	1	+	-	-	-
23*	Blood	135	16	4	4	0.25	0.12	0.12	2	0.12	0.5	+	-	-	bft-1
24*	Blood	120	256	8	8	0.25	0.25	0.25	1	0.06	1	+	+	-	-
25	Blood	122	32	16	8	8	4	8	1	4	0.5	-	-	+	-
26	Pus	149	256	8	64	0.25	0.5	0.25	0.06	8	0.25	+	+	-	bft-1
27	Tissue	136	32	8	4	0.25	0.25	0.12	1	4	1	+	-	-	-
28	Blood	141	16	4	8	0.25	0.25	0.12	256	0.12	0.5	+	-	-	-
29	Blood	143	32	4	4	0.25	0.25	0.12	0.06	0.06	1	+	-	-	-
30	Pus	11	256	8	64	0.5	1	2	1	2	0.5	+	+	-	-
31*	Blood	79	16	16	16	2	0.25	0.25	0.5	2	1	+	-	-	bft-1
32	Blood	149	256	8	32	0.25	0.25	0.25	256	0.06	0.25	+	+	-	bft-1
33	Blood	11	256	8	8	0.5	0.5	0.25	256	0.06	1	+	+	-	-
34*	Pus	40	256	8	32	1	0.5	0.5	0.5	4	0.5	+	+	-	-
35	Blood	11	8	4	2	0.12	0.25	0.12	1	0.06	1	+	-	-	-
36*	Blood	142	16	8	2	0.25	0.25	0.12	0.5	2	0.5	+	-	-	-
37	Tissue	12	256	128	256	2	2	4	256	4	0.25	+	+	-	-
38	Blood	149	8	4	4	0.12	0.25	0.12	256	8	0.5	+	-	-	bft-1
39	Blood	23	16	4	8	0.25	0.25	0.12	256	4	0.5	+	-	-	-
40	Tissue	87	256	4	16	0.5	0.25	0.25	256	8	0.5	+	+	-	-
Resistant/positive rate (%)			100	5.0	12.5	2.5	2.5	2.5	37.5	15.0	0	100	32.5	5.0	30.0

*Patients with a history of anaerobic antibiotic use within 1 yr prior to admission

†Patients who died due to infection with *B. fragilis*.

Abbreviations: MIC, minimum inhibitory concentration; PEN, penicillin; FOX, cefoxitin; CTT, cefotetan; PIP/TAZ, piperacillin-tazobactam; IMP, imipenem; CLN, clindamycin; MOX, moxifloxacin; MET, metronidazole.

patient infected with this isolate was an 88-yr-old woman with an intra-abdominal infection and bacteremia. She improved with metronidazole treatment for 9 days. She had been hospitalized within the past 3 months but had not used anaerobic antimicrobials within the past year. To the best of our knowledge, this was the first study to conduct an ST analysis of *B. fragilis* clinical isolates in Korea. Twelve isolates (30%) carried *bft*. Among the patients associated with these isolates, two were diagnosed as having colorectal malignancy; however, the influence of *bft* on colorectal carcinogenesis in these cases remains unclear. Continuous monitoring is necessary to detect resistant and virulent BFG clinical isolates.

In conclusion, recently isolated *B. fragilis* strains in Korea are of diverse STs. The emergence of highly carbapenem-resistant *B. fragilis* ST157 warrants caution. Understanding the genetic diversity and virulence profiles of *B. fragilis* can aid in developing targeted antimicrobial therapies and infection control measures, potentially improving patient outcomes and reducing mortality associated with these infections.

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AUTHOR CONTRIBUTIONS

Lee Y, Kim B, and Lee K designed the study. Lee Y and Kim M collected and identified the clinical isolates. Lee Y, Kim B, and Lee K performed the experiments and analyzed the data. Lee Y, Kim B, and Lee K contributed to the writing, editing, and review of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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