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Original Article

Molecular epidemiology and antimicrobial susceptibility profiles of *Campylobacter jejuni* isolated from bloodstream infections and enteritis in Japan

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ABSTRACT

Campylobacter enteritis (En) is the most frequently diagnosed bacterial En worldwide, including in Japan. *Campylobacter* spp. can also cause bloodstream infection (BSI), reactive arthritis, and Guillain-Barré syndrome. The purpose of this study was to clarify the characteristics of *Campylobacter jejuni* strains that cause BSI in comparison with En-causing strains. BSI strains (n = 40) and En strains that caused food poisoning (n = 67) were collected in Japan. Our study revealed that ST-4526 was predominant in BSI strains, and the overall distribution of sequence types was similar in both BSI and En strains. Differences in CPS type distribution might be related to the pathogenesis of bacteremia. Quinolone resistance rates were higher than those reported in previous studies, and strains resistant to both quinolones and tetracyclines were more frequently observed in BSI strains. Finally, we report a case of mixed infection with different STs in BSI.

1. Introduction

Campylobacter is a microaerobic gram-negative spiral rod-shaped or curved bacterium that primarily causes gastrointestinal disorders. Globally, the most common type of campylobacteriosis is that caused by *Campylobacter jejuni*. Major symptoms of campylobacteriosis include diarrhea, which ranges from loose to watery feces, as well as abdominal cramps and fever. In more severe cases, *Campylobacter* infection can lead to bloodstream infection (BSI), reactive arthritis, and Guillain-Barré syndrome (GBS), an autoimmune disease [1,2]. Skirrow et al. estimated the incidence of *Campylobacter* BSI to be approximately 1% in European reports [3]. According to data from the Ministry of Health, Labor and Welfare in Japan, *Campylobacter* food poisoning was the most common bacterial food poisoning in 2017, with 320 cases accounting for 32% of the total [4].

In recent years, highly pathogenic *C. jejuni* strains with region-specific genotypes have been reported, for example, sheep abortions caused by tetracycline-resistant *C. jejuni* ST-8 strains reported in the United States, and BSIs caused by serum-resistant *C. jejuni* ST-677 strains in Finland [5,6]. In Japan, ST-4526 (ST-21CC), which is resistant to quinolones, has been reported as the dominant type among isolates from food poisoning cases in recent years [7,8]. Although there are reports of *C. jejuni* ST-22 (HS19) strains that cause GBS in Japan, no reports have yet focused on the molecular epidemiology of *C. jejuni* BSI isolates [9,10].

The purpose of this study was to characterize the features of BSI-causing *C. jejuni* strains using molecular epidemiological analysis and antimicrobial susceptibility testing, and compare them with enteritis (En)-causing strains of *C. jejuni*. Clarifying the differences in molecular epidemiology and antimicrobial susceptibility profiles between BSI and En-causing strains in this study will lead to a review of the features of *C. jejuni* BSI.

2. Materials and methods

2.1. Bacterial collection and DNA extraction

Strains of *C. jejuni* that caused BSI (n = 40) were collected from eight hospitals in Japan. En-causing *C. jejuni* strains (n = 67), collected from local public health institutes in Nagoya city and Toyama prefecture in Japan, served as the experimental controls. Bacterial DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. To determine *Campylobacter* species, *C. jejuni/coli*-specific polymerase chain reaction (PCR) was performed [11,12].

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Table 1
Summary of bloodstream infection and enteritis-causing isolates of *Campylobacter jejuni* classified by multi-locus sequence type and capsular polysaccharide type.

Clonal complex	Sequence type	Bloodstream infection (n = 40)	%	Enteritis (n = 67)	%	Capsular polysaccharide type	
						Bloodstream infection (n = 40)	Enteritis (n = 67)
21		16	40.0	19	28.4	HS1 (1), HS2 (11), HS4 (3), HS8 (1)	HS1 (2), HS2 (9), HS53 (1), HS8 (7)
	19	0	0.0	4	6.0		HS1 (2), HS2 (1), HS53 (1)
	21	1	2.5	1	1.5	HS2 (1)	HS2 (1)
	50	1	2.5	2	3.0	HS1 (1)	HS8 (2)
	53	1	2.5	0	0.0	HS2 (1)	
	806	2	5.0	0	0.0	HS4 (2)	
	982	2	5.0	1	1.5	HS2 (2)	HS2 (1)
	2250	1	2.5	0	0.0	HS4 (1)	
	4253	0	0.0	1	1.5		HS2 (1)
	4526	7	17.5	5	7.5	HS2 (7)	HS2 (5)
	4562	0	0.0	1	1.5		HS8 (1)
	8075	0	0.0	2	3.0		HS8 (2)
	8122	1	2.5	0	0.0	HS8 (1)	
	10014	0	0.0	2	3.0		HS8 (2)
22	22	5	12.5	9	13.4	HS19 (5)	HS19 (9)
42		1	2.5	2	3.0	HS23 (1)	HS23 (1), UT[a] (1)
	42	1	2.5	1	1.5	HS23 (1)	HS23 (1)
	10410	0	0.0	1	1.5		UT[a] (1)
45		2	5.0	4	6.0	HS21 (2)	HS12 (1), HS21 (2), HS27 (1)
	45	0	0.0	1	1.5		HS12 (1)
	137	2	5.0	2	3.0	HS21 (2)	HS21 (2)
	3456	0	0.0	1	1.5		HS27 (1)
48	918	0	0.0	1	1.5		HS4 (1)
49		1	2.5	1	1.5	HS1 (1)	HS18 (1)
	9240	1	2.5	0	0.0	HS1 (1)	
	10854	0	0.0	1	1.5		HS18 (1)
52	52	0	0.0	1	1.5		HS5 (1)
61		2	5.0	0	0.0	HS4(2)	
	61	1	2.5	0	0.0	HS4 (1)	
	1244	1	2.5	0	0.0	HS4 (1)	
257	824	0	0.0	1	1.5		HS2 (1)
353	10425	0	0.0	2	3.0		HS3(2)
354		3	7.5	1	1.5	HS5(1), HS10 (1), HS53 (1)	HS10 (1)
	354	1	2.5	1	1.5	HS53 (1)	HS10 (1)
	10855	1	2.5	0	0.0	HS5 (1)	
	10858	1	2.5	0	0.0	HS10 (1)	
403	8264	0	0.0	2	3.0		HS23 (2)
443		4	10.0	1	1.5	HS37 (4)	HS37 (1)
	51	1	2.5	0	0.0	HS37 (1)	
	440	3	7.5	1	1.5	HS37 (3)	HS37 (1)
464		2	5.0	7	10.4	HS2 (1), HS8 (1)	HS1 (2), HS2 (1), HS8 (3), HS18 (1)
	4108	1	2.5	0	0.0	HS2 (1)	
	4389	1	2.5	5	7.5	HS8 (1)	HS1 (2), HS8 (3)
	5731	0	0.0	1	1.5		HS2 (1)
	10859	0	0.0	1	1.5		HS18 (1)
508	132	1	2.5	0	0.0	HS4 (1)	
574	305	0	0.0	1	1.5		HS5 (1)
607	607	0	0.0	1	1.5		HS53 (1)
Singleton		3	7.5	14	20.9	HS2 (1), HS3 (1), HS4 (1)	HS2 (1), HS3 (3), HS4 (1), HS8 (4), HS10 (2), HS15 (3)
	407	0	0.0	3	4.5		HS15 (3)
	468	1	2.5	0	0.0	HS4 (1)	
	922	1	2.5	3	4.5	HS3 (1)	HS3 (3)
	2274	0	0.0	1	1.5		HS8 (1)
	4618	0	0.0	1	1.5		HS8 (1)
	8071	0	0.0	2	3.0		HS10 (2)
	10849	0	0.0	1	1.5		HS2 (1)
	10850	0	0.0	1	1.5		HS4 (1)
	10851	0	0.0	1	1.5		HS8 (1)
	10853	1	2.5	0	0.0	HS2 (1)	
	10860	0	0.0	1	1.5		HS8 (1)

[a] UT = Undetermined type; specific amplification was not confirmed by capsular polysaccharide typing.

2.2. Multilocus sequence typing (MLST) analysis

PCR and sequence analysis for MLST were performed according to the PubMLST protocol [13]. The nucleotide sequence was assembled using ATGC software (GENETYX, Tokyo, Japan) and submitted to the PubMLST website (https://pubmlst.org/organisms/campylobacter-jejunicoli) to determine the allele number, sequence type (ST), and clonal complex (CC). If a novel allele sequence or a novel ST was found, it was registered in the PubMLST *C. jejuni/coli* database.

2.3. Capsular polysaccharide (CPS) region typing

Multiplex PCR for CPS typing was performed using bacterial DNA as a template and primer sets designed for the CPS region by Poly

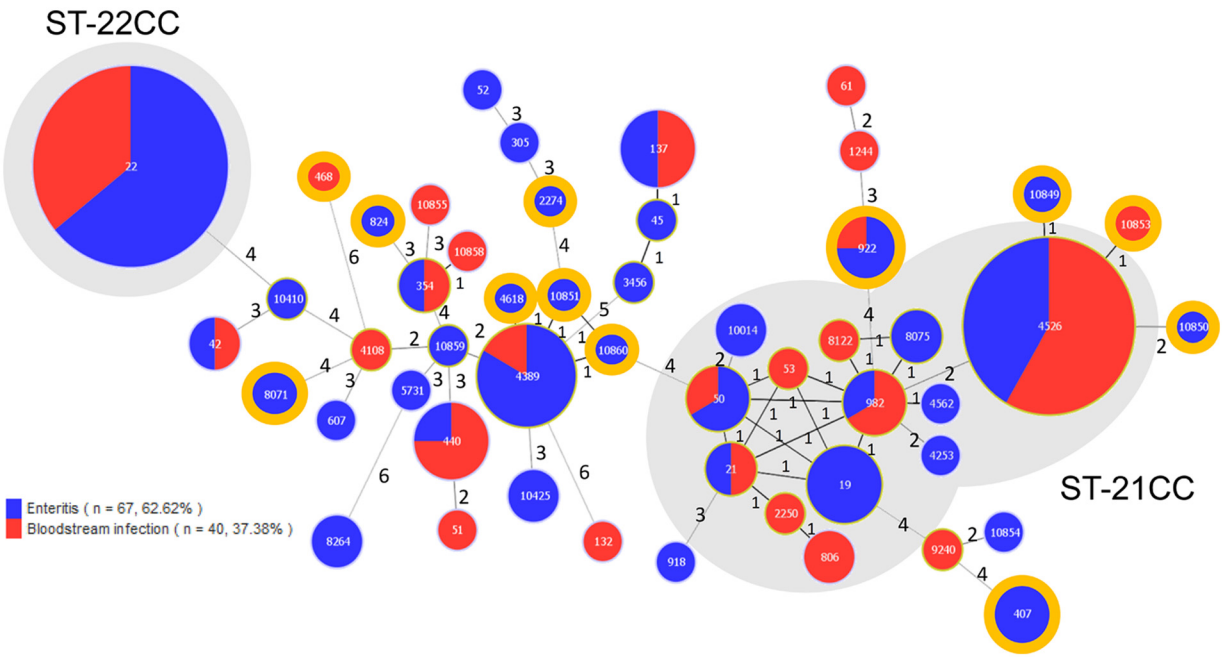

Fig. 1. Multilocus sequence type (MLST) distribution between bloodstream infection and enteritis-causing strains of *Campylobacter jejuni*. A minimum spanning tree was obtained by applying the goe-BURST distance method using PHYLOViZ 2.0 software. The red portion of the pie chart represents bloodstream infection-causing strains, and the blue portion enteritis-causing strains. The numbers indicate the difference between each sequence type (ST). The size of each circle represents the number of strains of each ST that were isolated. The circles with yellow borders indicate the undetermined clonal complex types (singletons) that were not in the PubMLST database.

et al. [14,15]. Multiplex PCR was performed using Ex Taq HS (TaKaRa, Shiga, Japan), and the amplicon was confirmed using a Multina Microchip Electrophoresis System (Shimadzu, Kyoto, Japan) with DNA 1000 kit (Shimadzu).

2.4. Antimicrobial susceptibility test

Antimicrobial susceptibility to nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin, erythromycin, and tetracycline was evaluated using the disk diffusion method with KB disk 'EIKEN' (Eiken Chemical, Tokyo, Japan). An additional antimicrobial susceptibility test was performed using Etest strips (bioMérieux, Tokyo, Japan) to determine the minimal inhibitory concentration (MIC) for cefepime (CFPM), imipenem (IPM), meropenem (MEPM), gentamicin (GM), and azithromycin (AZM). These schemes were based on the Clinical and Laboratory Standards Institute (CLSI) guidelines [16,17].

2.5. gyrA *sequencing*

To determine the mechanism of quinolone resistance, sequencing of the *gyrA* region of the quinolone resistance determinant region (QRDR) was performed [18]. The *gyrA* region was amplified using Ex Taq HS (TaKaRa), and sequencing was carried out with BigDye Terminator version 3.1 Ready Reaction Cycle Sequencing Kit (Thermo Fisher Scientific, Tokyo, Japan). The nucleotide sequence was assembled, translated into amino acid sequences, and compared with known sequences using GENETYX software (GENETYX).

2.6. Statistical analysis

Phylogenetic tree analysis was performed using PHYLOViZ 2.0 [19,20]. All statistical analyses were performed using RStudio [21,22]. Fisher's exact test was performed to compare the categorized values. Statistical significance was set at $P < 0.05$.

3. Results

MLST analysis of 107 isolates resulted in 17 CCs, including 51 STs, and 11 isolates were identified as novel STs: ST-10849, ST-10850, ST-10851, ST-10853, ST-10854 (ST-49CC), ST-10855 (ST-354CC), ST-10858 (ST-354CC), ST-10859 (ST-464CC), and ST-10860. The most frequently detected ST among the BSI strains was ST-4526, which accounted for 17.5%, followed by ST-22 (12.5%) and ST-440 (7.5%) (Table 1). The most frequently detected ST of the En strains was ST-22 (13.6%), followed by ST-4389 (7.6%) and ST-4526 (7.6%). There appeared to be no significant difference in the distribution of STs between BSI and En strains (Fig. 1). Furthermore, ST-51, ST53, ST-61, ST-132, ST468, ST-806, ST-2144, ST-2250, ST-4108, and ST-9240 were identified only in BSI strains.

Among the 17 CCs, the most frequently detected CC among the BSI strains was ST-21CC, which accounted for 40.0%, followed by ST-22CC (12.5%) and ST-443CC (10.0%). The most frequently detected CC among En strains was ST-21CC (28%), followed by ST-22CC (13.6%) and ST-464CC (10.4%). ST-61CC, including ST-61 and ST-1244, and ST-508CC, including ST-132, were identified only in BSI strains. Statistical analysis showed that the number of undetermined CC types (singletons) in En strains was significantly higher than in BSI strains ($P = 0.022$), although the genetic distributions of the singletons were scattered (Fig. 1).

The CPS types of 107 isolates were classified into 16 heat-stable antigen (HS) types (Table 1), with one isolate being undetermined. The most frequently isolated HS type in BSI strains was HS2, followed by HS4 complex, HS19, and HS37 (Fig. 2). Among the En strains, the HS8 complex was the most frequently isolated, followed by HS2 and HS19. Statistical analysis revealed that the HS4 complex was significantly more prevalent in BSI strains ($P = 0.013$), and the HS8 complex was significantly more prevalent in En strains ($P = 0.027$).

According to the results of the antimicrobial susceptibility test of 107 isolates, 33 isolates were susceptible to all antimicrobials tested in this study. Among the resistant strains, 63 isolates were resistant to quinolones and 44 isolates were resistant to tetracycline, of which 33 strains were resistant to both quinolones and tetracycline. No

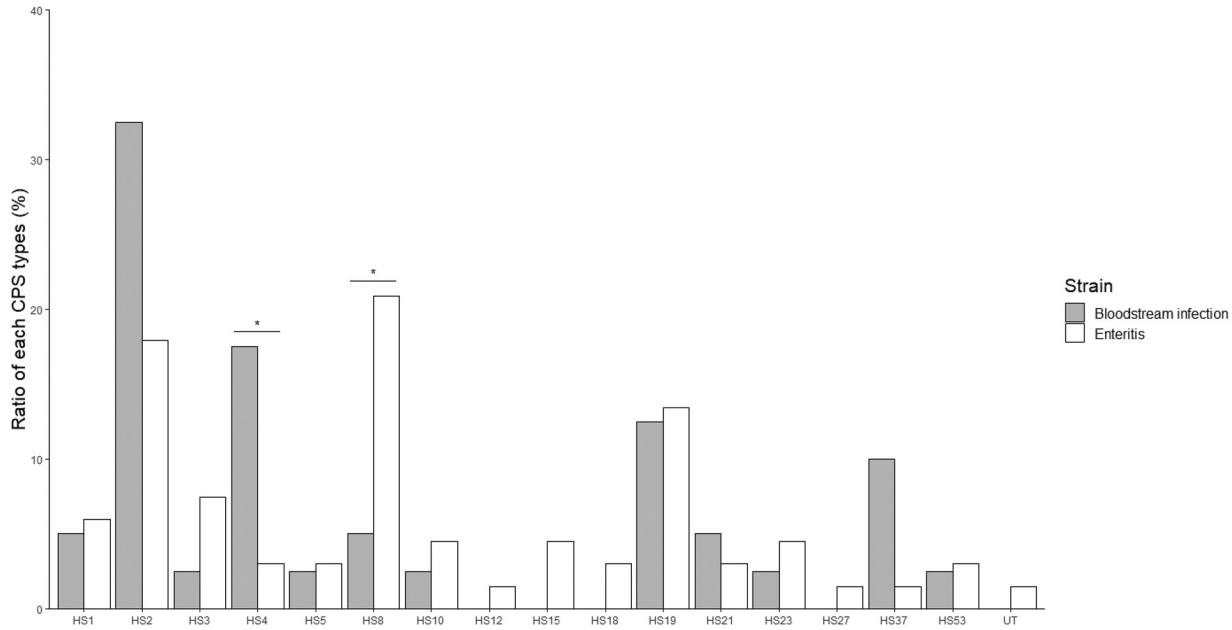

Fig. 2. Distribution of each capsular polysaccharide type in bloodstream infection-causing strains (gray bars) and enteritis-causing strains (white bars) of *Campylobacter jejuni* isolated in this study. UT = undetermined type; * Statistically significant differences between bloodstream infection and enteritis-causing strains were observed in HS4 complex ($P = 0.013$) and HS8 complex ($P = 0.027$).

macrolide-resistant strains were detected in this study. Sequencing analysis of the *gyrA* region revealed that 95.2% of the quinolone-resistant strains harbored the Thr-86-Ile mutation in the QRDR.

Among the BSI strains, 15 isolates (37.5%) were susceptible to all the tested antimicrobials, 24 isolates (60.0%) were resistant to quinolones, 17 isolates (42.5%) were resistant to both quinolones and tetracycline, and one isolate (2.5%) was resistant to tetracycline (Fig. 3). Among the En strains, 18 (26.9%) were susceptible and 39 (58.2%) were resistant to quinolones, 16 (23.9%) were resistant to both quinolones and tetracycline, and 10 isolates (14.9%) were resistant to tetracycline.

The prevalence of both quinolones and tetracycline resistance tended to be higher in the BSI strains, although no statistically significant difference was observed ($P = 0.053$). On the other hand, the

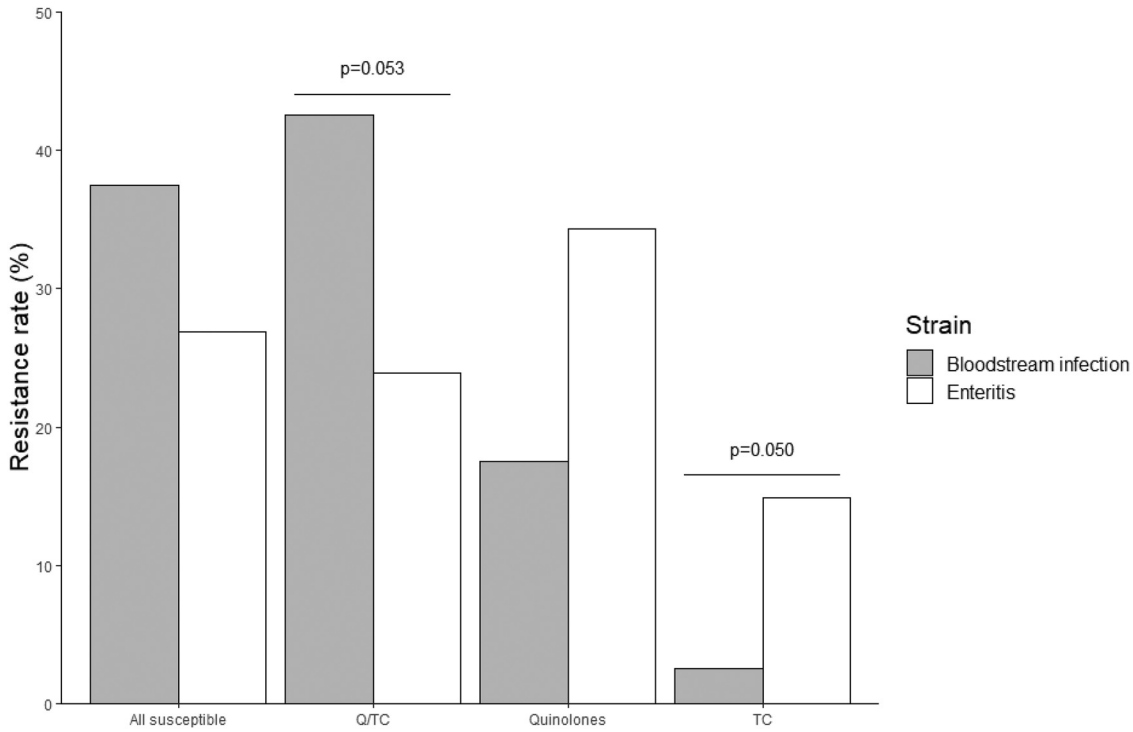

Fig. 3. Antimicrobial susceptibility of bloodstream infection-causing strains (gray bars) and enteritis-causing strains (white bars) of *Campylobacter jejuni*. "All susceptible" indicates susceptibility to all tested quinolones, tetracycline, and erythromycin. "Q/TC" indicates resistance to both quinolones and tetracycline. "Quinolones" indicates resistance to only quinolones. "TC" indicates resistance to only tetracycline.

number of BSI strains resistant to tetracycline was significantly lower than the number of En strains (Fig. 3). The distribution of antimicrobial susceptibility profiles for each genotype varied, and no significant correlation was found. The MICs of CFPM, IPM, MEPM, GM, and AZM were determined using Etest strips (Table 2). MICs of IPM, MEPM, GM, and AZM were distributed within a considerably low range, indicating that possible candidates for the treatment of BSI caused by *C. jejuni* are considered to be effective.

Isolates showing different antimicrobial susceptibility profiles were identified in a single patient with BSI (Supplementary Table). One isolate was resistant to quinolones, and the other was resistant to both quinolones and tetracycline. These isolates showed differences in MLSTs and CPS types. These results account for a rare case of BSI with a mixture of *C. jejuni* isolates with diverse genetic backgrounds.

4. Discussion

In this study, we analyzed 40 *C. jejuni* strains isolated from BSI cases using molecular epidemiological methods, such as MLST analysis and CPS typing, in addition to antimicrobial susceptibility tests, and compared them with 67 *C. jejuni* strains isolated from food poisoning cases. Overall, the lack of significant differences in the distribution of genotypes in MLST between BSI and En strains suggests that *C. jejuni* BSI may mostly occur secondary to *C. jejuni* En caused by contaminated food. ST-4526 was the most frequently identified ST among BSI strains (17.5%), which was recently identified as the dominant type among En strains in Japan [7,8].

Previous study showed that CPS is associated with the development of bacteremia in mice [23]. Multiplex PCR was performed to identify CPS types, and it showed that HS2, HS4 complex, and HS19 accounted for 62.5% of all BSI strains, and HS8 complex, HS2, and HS19 accounted for 52.2% of all En strains, which is similar to reports from Bangladesh and Peru that noted the predominance of these particular CPS types [24–26]. The HS4 complex was significantly more frequently identified in BSI strains than in En strains, which is consistent with previous reports [3]. In Finland, ST-677, which is associated with the HS4 complex, was reported to be highly pathogenic and a frequent cause of bacteremia [27]. These results strongly support that the HS4 complex is associated with BSI, although no specific ST was associated with the HS4 complex in this study. On the other hand, the HS8 complex was less frequently isolated in bacteremia strains. One of the reasons for the reduced incidence of bacteremia associated with the HS8 complex may be a deficit in the *O*-methyl phosphoramidite synthase region in the HS8 complex, which is associated with serum resistance [14].

Recently, the quinolone resistance rate has been increasing in *Campylobacter* spp. [28]. The mechanism of quinolone resistance in *C. jejuni* has been reported to be due to point mutations in the *gyrA* region, but not in *parC* and *gyrB* [8,29,30]. In this study, the quinolone-resistance rate among *C. jejuni* strains was approximately 60%, which was higher than that reported in previous studies [8,31,32]. BSI strains tended to be more resistant to both quinolones and tetracycline than the En strains, although the difference was not statistically significant. The Thr-86-Ile mutation in QRDR was the major quinolone resistance mechanism in both the BSI and En strains. No macrolide-resistant strains were identified, in accordance with previous reports from Japan [8].

Antimicrobial treatment is not usually necessary for campylobacteriosis, but it may be essential in some cases, such as BSI. In addition, *C. jejuni* is naturally resistant to cephalosporins; therefore, it is not recommended for treating patients with systemic symptoms [33,34]. The MICs of IPM, MEPM, GM, and AZM, which were determined using Etest strips, were considerably low, encouraging further clinical studies to determine the efficacy of these antibiotics in the treatment of *C. jejuni* BSI.

Table 2
Distribution of minimum inhibitory concentrations of bloodstream infection-causing *Campylobacter jejuni* strains against 5 types of antiobiotics.

Antibiotics	No. of strains tested	Range	50%	90%	Minimum inhibitory concentration (μg/mL)																		
					0.004	0.008	0.012	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.190	0.250	0.380	0.500	0.750	1.000	1.500	2.000	3.000	4.000	6.000
Cefepime	40	0.5-6.0	1.5	3.0														2	2	13	13	5	3	1	1
Imipenem	40	0.064–0.190	0.125	0.125								10	19	10	1										
Meropenem	40	0.004–0.125	0.016	0.064	1	9	8	7	6	2	2	4		1											
Gentamicin	40	0.125–0.750	0.25	0.50										6	7	13	7	3	4						
Azithromycin	40	0.047–0.380	0.094	0.19							5	13	5	8	7	1	1								

In the present study, a BSI case was identified in which blood culture yielded two distinct *C. jejuni* strains with different genetic backgrounds and antimicrobial susceptibility profiles. MLST analysis showed that one strain resistant to quinolone only was classified as ST-22 (HS-19), while the other, resistant to both quinolones and tetracycline, was classified as ST-10853 (HS-2). A previous study showed mixed infection caused by multiple serotypes of *C. jejuni* in a case of En [35] and this is the first BSI case caused by multiple serotypes and genotypes of *C. jejuni* strains.

This study has some limitations. The first is the small sample size due to the low incidence of BSI caused by *C. jejuni*. Second, there is a difference in the geographical distribution of the institutions where the BSI and En strains were collected, which may influence the distribution of strains themselves. Third, this study did not analyze serum resistance, which may be associated with BSI. Finally, we did not use patient information for this study.

5. Conclusions

The present study compared the molecular epidemiology of *C. jejuni* strains isolated from BSI and En cases in Japan. The results of MLST analysis were similar, and ST-4526 was the dominant type in both strains. CPS types indicated that the Penner serotypes, HS2, HS4 complex, and HS19, were frequently identified in BSI strains, while the HS8 complex was less associated with BSI. Quinolone resistance was predominant in Japanese *C. jejuni* isolates, and resistance to both quinolone and tetracycline was predominant in BSI strains. Furthermore, a rare case of BSI caused by different serotypes and genotypes of *C. jejuni* strains was identified. Further investigation is necessary to elucidate the clinical characteristics of *C. jejuni* infections, including BSI, and to clarify the relationship between clinical aspects and causative *Campylobacter* strains.

Authors' contribution

TY contributed to the conceptualization and funding acquisition, methodology and review and editing, revising of this study; YK contributed to data curation and formal analysis, investigation, methodology, writing original draft, revising of this study; SS reviewed the manuscript. All authors read and approved the final manuscript.

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Conflict of interests

The authors declare that they have no conflict of interests.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2022.115681.

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