Short Communication

Genetic Diversity of *Campylobacter jejuni* Isolates from Korea and Travel-Associated Cases from East and Southeast Asian Countries

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SUMMARY: Forty domestic and travel-associated *Campylobacter jejuni* isolates were analyzed by profiling 7 pathogenic genes (*cdtB*, *cadF*, *Cj0131*, *ciaB*, *racR*, *wlaN*, and *virB11*) along with multilocus sequence typing (MLST) and antimicrobial susceptibility testing. *cdtB*, *cadF*, and *Cj0131* were present in all isolates, whereas *virB11* was not detected in either domestic or travel-associated isolates. *ciaB* was present in all domestic isolates and 94% of travel-associated isolates. The respective detection rates of *racR* and *wlaN* in domestic and travel-associated isolates were 94% and 71% and 35.3% and 23%, respectively. MLST analyses of the 40 isolates generated 25 different sequence types (STs). ST-443 (12 isolates) and ST-21 (8 isolates) were dominant among the domestic isolates; however, STs varied among travel-associated isolates. Nalidixic acid, tetracycline, and ciprofloxacin resistance rates of the 40 isolates were 100% (40/40), 95% (38/40), and 88% (35/40), respectively. Domestic isolates exhibited 2-fold higher ciprofloxacin, telithromycin, and chloramphenicol resistance rates than travel-associated isolates. These results indicate a diverse genetic background for travel-associated *C. jejuni* and suggest that this pathogen may be an important emerging public health threat to travelers.

Campylobacter jejuni is a curved, gram-negative, microaerobic bacterium and an important etiological agent of human diarrhea. Campylobacteriosis is a leading cause of traveler's diarrhea, particularly among individuals returning from East Asian countries (1). *Campylobacter* spp. comprise a common cause of human bacterial enteritis in Korea, although human campylobacteriosis cases may have been under-reported (2). Previous studies have mainly addressed genetic relationships and antibiotic resistance among *C. jejuni* isolates from chickens and humans in Korea and other East Asian countries (3,4). However, a comparison of the genetic relationships and antibiotic resistance among domestic and travel-associated *C. jejuni* isolates from East Asian countries has not yet been published. Therefore, in this study, we investigated the pathogenic gene profiles, multilocus sequence typing (MLST) results, and antimicrobial susceptibilities of *C. jejuni* isolates from travelers who returned to Korea in comparison with those from domestically acquired cases.

A total of 20,905,534 travelers enter Korea through the Incheon International Airport in 2012. Of these, 9,796 (0.05%) exhibit symptoms of diarrhea; travelers' diarrhea was further diagnosed in 3,999 patients. An

analysis of the stool samples from the 3,999 patients recovered 80 *C. jejuni* isolates. Seventeen of the 80 *C. jejuni* isolates (i.e., a travel-associated set; individuals had traveled overseas for <3 weeks) were collected from diarrheal travelers who were reported to have been away from Korea for <3 weeks. The travel destinations of these patients were East Asian countries, including Indonesia, Philippines, and China. Another set of 23 isolates (domestic set) was obtained from patients with diarrhea who were confirmed to have experienced no international travels within the previous 2–3 years and no family members who had recently traveled internationally; all resided in metropolitan Seoul. Forty *C. jejuni*-identified strains were collected from individuals in 2012. These 40 individuals were 20–30-year-old adults with diarrhea (domestic isolates were collected from 9 males and 14 females; travel-associated isolates were collected from 9 males and 8 females). All strains were incubated on Muller–Hinton agar (Oxoid, Nepean, ON, Canada) at 42°C under microaerobic conditions (85% N₂, 5% O₂, 10% CO₂) for 48 h. Template DNAs for PCR were extracted via the conventional boiling method. Fresh *C. jejuni* cultures were suspended in 300 µl TE buffer, and the suspensions were boiled at 95°C for 10 min. PCR was performed using the Expanded High Fidelity Polymerase System (Roche, Basel, Switzerland) or *Taq* polymerase (Takara, Shiga, Japan) according to the manufacturer's instructions. The PCR conditions were 94°C for 5 min; 35 cycles of 94°C for 30 s; 50°C for 30 s; and 72°C for 1 min; and finally, 72°C for 5 min.

cadF and *racR*, *ciaB* and *virB11*, *cdtB*, and *wlaN* were selected as pathogenic genes responsible for the induc-

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tion of colonization (5,6), invasion (7,8), toxin production (9), and ganglioside expression mimicking Guillain-Barré syndrome (8,10), respectively. *Cj0131* is another putative virulence gene (unpublished data) that encodes a putative M23 family peptidase in *C. jejuni*. It was also selected in this study. *cadF*, *cdtB*, and *Cj0131* were detected in all isolates. The *ciaB*, *racR*, and *wlaN* detection rates varied among the travel and domestic isolates, and *virB11* was not detected in any isolate (Table 1). *ciaB* was present in 100% of the domestic isolates and 94% of the travel-associated isolates. The respective *racR* and *wlaN* gene detection rates were 94% and 71% and 35.3% and 23% for the domestic and

travel-associated isolates, respectively, and the intergroup differences were not significant. The virulence gene distribution differs widely between countries, possibly because of differences in the food consumption and eating habits (11). The higher distributions of *ciaB*, *racR*, and *wlaN* among domestic isolates were similar to those in a previous report from Japan (12).

MLST provides an index of variations in housekeeping genes and is useful for investigating the DNA sequence diversity (13). MLST amplifies a segment of 7 housekeeping genes: *aspA* (aspartate ammonia-lyase, 477 bp), *atpA* (or *uncA*, ATP synthase F1 sector, alpha subunit, 489 bp), *glnA* (glutamine synthetase, 477 bp),

Table 1. Presence of pathogenic genes, serotype, antimicrobial resistance profile and MLST types of domestic and travel-associated isolates

Work field	Isolated ID	<i>cadF</i>	<i>Cj0131</i>	<i>cdtB</i>	<i>ciaB</i>	<i>racR</i>	<i>wlaN</i>	<i>virB11</i>	Serotype	Antimicrobial resistance profile	ST
Travel-associated	I-88 Indonesia	+	+	+	+	+	+	–	1	CIP, TE, NA	ST-6098
	I-89 Indonesia	+	+	+	+	+	–	–	4	CIP, TE, NA	ST-6099
	I-474 Indonesia	+	+	+	+	–	–	–	15	CIP, TE, NA	ST-6102
	I-623 Indonesia	+	+	+	+	–	–	–	4	CIP, TE, NA	ST-6104
	I-730 Indonesia	+	+	+	+	+	–	–	UT	CIP, NA	ST-6106
	I-731 Indonesia	+	+	+	+	+	–	–	23	CIP, NA	ST-6107
	I-90 Philippines	+	+	+	+	+	–	–	UT	CIP, TE, NA	ST-6100
	I-92 Philippines	+	+	+	+	+	+	–	52	CIP, TE, NA	ST-353
	I-467 Philippines	+	+	+	+	–	+	–	UT	CIP, TE, NA	ST-6100
	I-470 Philippines	+	+	+	+	+	–	–	UT	TE, NA	ST-257
	I-625 Philippines	+	+	+	+	+	–	–	4	TE, NA	ST-6105
	I-20 China	+	+	+	+	+	–	–	4	CIP, TE, NA	ST-6096
	I-91 China	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	I-86 China	+	+	+	+	+	–	–	21	CIP, NA	ST-6097
	I-478 China	+	+	+	+	–	–	–	6	CIP, NA	ST-6103
	I-93 China	+	+	+	–	+	–	–	52	CIP, TE, NA	ST-6101
	I-257 China	+	+	+	+	+	+	–	2	CIP, TE, NA	ST-464
Positive* (%)		100	100	100	94	71	23	0			
Domestic	D-4	+	+	+	+	+	+	–	2	CIP, TE, NA	ST-21
	D-6	+	+	+	+	+	+	–	2	CIP, TE, NA	ST-21
	D-55	+	+	+	+	+	+	–	2	CIP, TE, NA	ST-21
	D-105	+	+	+	+	+	–	–	15	CIP, TE, NA	ST-658
	D-127	+	+	+	+	–	–	–	12	CIP, TE, NA	ST-45
	D-139	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-163	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-165	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-166	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-167	+	+	+	+	+	–	–	37	CIP, NA	ST-443
	D-170	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-171	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-173	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-174	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-176	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-443
	D-200	+	+	+	+	+	+	–	2	CIP, TE, NA	ST-443
	D-590	+	+	+	+	+	–	–	3	CIP, TE, NA	ST-353
	D-596	+	+	+	+	+	+	–	37	CIP, TE, NA	ST-21
	D-597	+	+	+	+	+	+	–	37	CIP, TE, NA	ST-21
	D-600	+	+	+	+	+	+	–	2	CIP, TE, NA	ST-21
	D-601	+	+	+	+	+	+	–	2	CIP, TE, NA	ST-21
	D-606	+	+	+	+	+	+	–	4	CIP, TE, NA	ST-607
	D-702	+	+	+	+	+	–	–	37	CIP, TE, NA	ST-21
Positive* (%)		100	100	100	100	94	35.3	0			

*, $P < 0.05$ by chi-square test.

UT; untypeable, CIP; ciprofloxacin, TE; tetracycline, NA; nalidixic acid.

glcA (citrate synthase, 402 bp), *glyA* (serine hydroxyl methyl transferase, 507 bp), *pgm* (phosphoglucose mutase, 498 bp), and *tkt* (transketolase, 459 bp) to yield a total composite sequence length (all 7 loci) of 3,309 bp. MLST was performed for all strains using primer sets described elsewhere (14). The alleles and sequence types (STs) were identified using the Perl program MLSTparser3 (15). Novel alleles and STs were submitted to the PubMLST *C. jejuni*/*E. coli* databases (<http://pubmlst.org/campylobacter/>). The 40 *C. jejuni* isolates were assigned to 25 clonal complexes. The most commonly identified clonal complex was ST-443 (12 isolates, including 11 domestic isolates and 1 travel-associated isolate), followed by ST-21 (8 domestic isolates), whereas 12 STs were new alleles (Table 1). Most domestic isolates shared ST-443 and ST-21. ST-443 has been widely detected in isolates including those from poultry, environment, and humans (16). Also, ST-21 and ST-45 have been the predominant type among chicken, turkey, and human isolates from sporadic cases (17). STs of the travel-associated isolates exhibited a diverse pattern. New STs (ST-6096 to ST-6107) were identified in 12 of 17 travel-associated isolates. A phylogenetic analysis based on the *C. jejuni* STs was conducted, and the diverse strains clustered with the reference genotypes as shown in the tree, thus confirming the comparative sequence analyses in a tree drawing (<http://pubmlst.org/cgi-bin/mlstanalyse/mlstanalyse.pl?site=pubmlst&page=treedraw&referer=pubmlst.or>). In the phylogenetic analysis, the domestic isolates were divided into 2 groups comprising 11 domestic isolates (ST-443, ID: D-139, 163, 165, 166, 167, 170, 171, 173, 174, 176, and 200) and 8 domestic isolates (ST-21, ID: D-4, 6, 55, 596, 597, 600, 601, and 702). The travel-associated isolates were very diverse (Fig. 1).

Serotyping according to the Penner HS method has been successfully used to identify *C. jejuni* subtypes

(Denka Seiken Japan, Tokyo, Japan) (18). However, the Penner HS method, which involves an antisera kit, yields complex results. Furthermore, PCR methods that can detect the major *C. jejuni* capsular types are also available for determining *C. jejuni* serotypes. Additionally, the PCR-based method can distinguish the serotype complexities (e.g., HS4 and HS4/13/64) (19). In the present study, the PCR-based method was able to subtype 90% (36/40) of the isolates. Eleven serotypes were identified among the 40 isolates. The domestic isolates included serotypes 37 ($n = 13$), 2 ($n = 6$), 12 ($n = 1$), 15 ($n = 1$), 3 ($n = 1$), and 4 ($n = 1$). The travel-associated isolates included serotypes 4 ($n = 4$), 52 ($n = 2$), 1 ($n = 1$), 21 ($n = 1$), 23 ($n = 1$), 2 ($n = 1$), 37 ($n = 1$), 15 ($n = 1$), and 6 ($n = 1$) as well as untypeable (UT, $n = 4$) (Table 1). Among the 40 isolates, the predominant serotype was 37, followed by serotypes 2, 4, and 52. The most commonly identified ST-443 and ST-21 serotypes were 37 and 2, respectively. The newly identified clonal complexes (ST-6096 to ST-6107) exhibited various serotypes (1, 4, 6, 15, 21, 23, 52, and UT). Among these, ST-6096, ST-6099, ST-6104, and ST-6105 exhibited serotype 4. The 2 major serotypes among the domestic isolates were 37 (13 isolates) and 2 (6 isolates), but no major serotypes were identified among the travel-associated isolates. The trends of the serotyping and MLST results were similar.

The minimum inhibitory concentrations (MICs) of the 40 isolates for 8 antibiotics (gentamicin, nalidixic acid, ciprofloxacin, telithromycin, azithromycin, erythromycin, chloramphenicol, and tetracycline) were determined using Sensititre (TREK Diagnostic Systems, Cleveland, OH, USA) (20). All tested isolates were resistant to nalidixic acid and highly resistant to tetracycline and ciprofloxacin, with respective resistance scores of 100% (40/40), 95% (38/40), and 88% (35/40) when tested according to the CLSI guideline (21). All tested isolates were susceptible to erythromycin, chloramphenicol, telithromycin, gentamycin, and azithromycin. When the MICs for 90% of the isolates were compared (MIC₉₀), the values associated with ciprofloxacin, telithromycin, and chloramphenicol were 2-fold higher for domestic isolates than for traveler-associated isolates. Furthermore, the lower limits of the domestic isolates for ciprofloxacin, telithromycin, tetracycline, and streptomycin were more than 2-fold higher. The MIC₅₀ and MIC₉₀ values for each antibiotic are shown in Table 2. In Korea and other East Asian countries, quinolones and tetracycline are the most widely used antimicrobials in veterinary settings (22,23). In our results, *C. jejuni* from domestic isolates had higher antimicrobial resistance rates than did travel-associated isolates. Additionally, a higher incidence of antimicrobial resistance among *Campylobacter* spp. isolates was found in chicken meat from Korea than in meat from other East Asian countries (4,24), with ciprofloxacin-nalidixic acid-tetracycline resistance being the most frequent resistance profile. Hong et al. (24) reported that 93.4% of the *Campylobacter* spp. isolated from chicken meats in Korea showed this antibiotic resistance triad pattern. As the information regarding *C. jejuni* isolates from Indonesia and Philippines are possibly out of date (21,22), our data may not be representative of *C. jejuni* isolates from East Asian countries (25,26). Most of the *C. jejuni*

Fig. 1. Phylogenetic analysis of 40 *Campylobacter jejuni* isolates.

Table 2. Minimum inhibitory concentrations of 40 *Campylobacter jejuni* isolates

Antibiotic	MIC range ($\mu\text{g/ml}$)		MIC ₅₀ ($\mu\text{g/ml}$) ¹⁾		MIC ₉₀ ($\mu\text{g/ml}$) ²⁾	
	Travel-associated	Domestic	Travel-associated	Domestic	Travel-associated	Domestic
Gentamicin	0.5–1	0.25–1	0.5	0.5	1	1
Nalidixic acid	64	64	64	64	64	64
Ciprofloxacin	0.12–32	8–64	16	16	32	64
Telithromycin	0.25–1	0.5–2	0.5	0.5	1	2
Azithromycin	0.03–0.06	0.03–0.06	0.03	0.03	0.06	0.06
Erythromycin	0.25–1	0.25–1	0.5	0.5	1	1
Chloramphenicol	1–2	0.5–4	2	2	2	4
Tetracycline	0.25–64	4–64	64	64	64	64
Streptomycin	0.5–2	1–2	1	2	2	2

¹⁾: MIC₅₀, minimum inhibitory concentration of 50%.

²⁾: MIC₉₀, minimum inhibitory concentration of 90%.

isolates both from domestic and travel-associated patients were sensitive to erythromycin, the antimicrobial agent used to treat human campylobacteriosis.

In conclusion, the pathogenic gene distribution and antibiotic resistance rates were higher among domestic isolates than among travel-associated isolates, although the *C. jejuni* strains isolated from travelers also caused diarrheal disease. It follows that, probabilistically, these untyped strains are more likely to associate with regionally untested antigens than with widespread strains and that acquired protection may therefore be ineffective upon exposure to uncommon strains, as shown in a Canadian study (27,28). Additionally, the diverse genetic backgrounds of *C. jejuni* isolates from travel-associated subjects suggest that *C. jejuni* could be an important emerging public health threat to travelers. Antimicrobial resistance and multidrug resistance in *C. jejuni* should be continuously monitored, and further epidemiologic research to understand the possible transmission of *C. jejuni* is required.

The genetic background of travel-associated campylobacteriosis differs from that of domestically acquired infections, and returning travelers may harbor multiple exotic strains that could subsequently spread to domestic populations despite limited person-to-person transmission. Special attention should be paid to travelers, and the results presented herein may offer potentially useful information for international travelers, clinicians, public health officials, travel agencies, and the larger international travel community.

Conflict of interest None to declare.

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