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First Report and Molecular Characterization of a *Campylobacter jejuni* Isolate with Extensive Drug Resistance from a Travel-Associated Human Case

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Campylobacter is a common causative agent of bacterial diarrhea worldwide (1). Uncomplicated illnesses caused by *Campylobacter* spp. are characterized by symptoms such as fever, abdominal cramping, and diarrhea; they are usually self-limiting and do not require antimicrobial treatment. However, cases of severe, prolonged, or relapsing illness require antimicrobial treatment. Macrolides and fluoroquinolones are the first- and second-choice antimicrobials for treating *Campylobacter* infections (2). Furthermore, severe systemic infections require the use of an aminoglycoside such as gentamicin. Increased antimicrobial resistance to erythromycin, fluoroquinolones, and tetracycline has been reported in both humans and animals in many countries (3), and it could compromise the treatment of human infections. Especially because of the rapid increase in global travel, concerns about pathogenic bacteria derived from international travel have increased (4).

As part of the national surveillance for food-borne disease in South Korea, a total of 4,788 human diarrheal stool samples collected from January 2007 through December 2009 were examined and 154 *Campylobacter* isolates were obtained. In a previous article (5), we reported the global antimicrobial susceptibilities to seven antimicrobials of 121 of these 154 isolates (the remaining 33 isolates failed to grow after transfer from stock storage). In this work, we focused on *C. jejuni* CCARM 13322, which was originally isolated from a 21-year-old Korean woman who visited a public health center in Seoul because of fever and diarrhea after traveling to the Philippines in 2008.

The agar dilution method, in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (6, 7), was used to determine the MICs of the antimicrobials amikacin, ampicillin, azithromycin, cefotaxime, ceftazidime, chloramphenicol (Fluka, Buchs, Switzerland), ciprofloxacin (Korea Research Institute of Chemical Technology, Daeduk, South Korea), clindamycin, enrofloxacin (Dr. Ehrenstofer, GmbH, Augsburg, Germany), erythromycin, gentamicin, meropenem, nalidixic acid, streptomycin, and tetracycline. All of the chemicals used in this study were purchased from Sigma (St. Louis, MO) unless otherwise stated. Isolates were inoculated onto Müller-Hinton agar (BBL) containing 5% sheep blood and incubated for 48 h under microaerobic conditions at 36°C in a GasPak jar with a GasPak EZ Campy Container System (Becton, Dickinson, Sparks, MD) with *Campylobacter jejuni* ATCC 33560 as a control. CLSI-approved MIC quality control limits for fastidious organisms were used for the control of agar dilution performance for ciprofloxacin, erythromycin, and gentamicin. The MIC interpretive standards of *Staphylococcus* spp. and veterinary pathogens defined by the CLSI were employed as breakpoints for erythromycin and enrofloxacin, respectively (8). For the MIC breakpoints of the other antimicrobials, the MIC interpretive standards for *Enterobacteriaceae* were utilized (7).

Genomic DNA of *C. jejuni* was extracted with a G-spin genomic extraction kit (Intron Biotechnology, Seoul, South Korea). Seven genes responsible for antimicrobial resistance [bla_{TEM}, *cmeB*, gyrase A, *tet*(O), 23S rRNA, integrase, class I integron gene cassettes] were detected by PCR. The sequences of the primers used for each gene, the sizes of the PCR products obtained, and the PCR conditions used are presented in Table 1. PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced by Bionics (Seoul, South Korea). DNA sequences were compared against a database by using the online

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TABLE 1 Primers used for PCR of antimicrobial resistance genes

Gene	Primer (5′→3′)		Annealing temp (°C)	Product length (bp)	Reference
	Forward	Reverse			
bla_{TEM}	ATAAAATTCTTGAAGACGAAA	GACAGTTACCAATGCTTAATC	54	1,100	17
cmeB	ATTTCAGGATTTGTTGCTCT	GAACCACCCTTGATACAAGT	54	705	18
gyrA	ATTTTTAGCAAAGATTCTGAT	CCATAAATTATTCCACCTGT	50	673	This study
tet(O)	GGCGTTTTGTTTATGTGC	ATGGACAACCCAACAGAAGC	53	559	This study
23S rRNA	TGATCGAAGCCCGAGTAAAC	CCAGACATTGTCCCACTTGA	52	353	18
Integrase	TGCGGGTYAARGATBTKGATTT	CARCACATGCGTRTARAT	55	491	19
Class I integron	GGCATCCAAGCAGCAAG	AAGCAGACTTGACTCGA	55	Variable	20

TABLE 2 Antimicrobial susceptibilities of *C. jejuni* CCARM 13322

Antimicrobial category	Antimicrobial agent	MIC (μg/ml)		MIC[a]	Interpretation[b]
		C. jejuni ATCC 33560	*C. jejuni* CCARM 13322		
Penicillins	Ampicillin	4	>128	≥32	R
Phenicols	Chloramphenicol	1	32	≥32	R
Fluoroquinolones	Ciprofloxacin	≤0.5	32	≥4	R
	Enrofloxacin	≤0.5	8	≥2	R
	Nalidixic acid	8	128	≥32	R
Macrolides	Azithromycin	0.06	>128	≥8	R
	Erythromycin	2	>128	≥32	R
Lincosamides	Clindamycin	0.5	64	≥4	R
Aminoglycosides	Gentamicin	1	>128	≥8	R
	Amikacin	16	128	≥32	R
Tetracyclines	Tetracycline	1	128	≥16	R
Extended-spectrum cephalosporins	Cefotaxime	8	16	≥64	I
	Ceftazidime	16	32	≥32	R
Streptomycins	Streptomycin	8	128		R
Carbapenems	Meropenem	0.015	0.25	≥16	S

[a] Criteria for resistance according to the MIC interpretive standards of infrequently isolated or fastidious bacteria defined by the CLSI were employed as breakpoints for ciprofloxacin, erythromycin, and tetracycline. For enrofloxacin, MIC interpretive standards for veterinary pathogens were employed. For other antimicrobials, MIC interpretive standards for *Enterobacteriaceae* and *Staphylococcus* spp. were utilized.
[b] R, resistant; I, intermediate; S, susceptible.

BLAST algorithm at the National Center for Biotechnology Information Web server (http://www.ncbi.nlm.nih.gov).

The supernatant of crude bacterial cell extract was analyzed by isoelectric point focusing (IEF) gel electrophoresis, and enzyme activities were detected by overlaying the gel with 500 μg/ml nitrocefin (Oxoid, Basingstoke, United Kingdom) with IEF standards (9). Extended-spectrum β-lactamase (ESBL) production was detected with the clavulanate double-disk synergy test (DDST) (9), and diameters of growth inhibition zones were measured after 48 h of incubation at 37°C under microaerobic conditions. *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *C. jejuni* ATCC 33560 were used as controls for DDST. The ESBL phenotype was defined as an increase of ≥5 mm in the diameter of the inhibition zone around a clavulanate-containing disk compared to the zones of corresponding disks without clavulanate.

C. jejuni CCARM 13322 was classified by multilocus sequence typing (MLST) as a novel sequence type, ST5075, by PubMed (http://pubmlst.org/campylobacter/), and it was resistant to 14 antimicrobials but not meropenem (Table 2). *C. jejuni* CCARM 13322 possessed a Thr86Ile mutation in the quinolone resistance-determining region (QRDR) of *gyrA* and an A2075G mutation in the peptidyl transferase region in domain V of the 23S rRNA that is responsible for macrolide resistance (Table 3). In addition to these mutations, *tet*(O), which is responsible for tetracycline resistance, was detected. An ESBL with a pI of 6.2 was observed with nitrocefin by IEF and DDST of ceftazidime and cefotaxime with clavulanate. When an efflux assay was performed as previously reported (10), efflux was not observed even though an efflux gene for fluoroquinolones, *cmeB*, was detected by PCR. These findings demonstrated that the fluoroquinolone resistance of *C. jejuni* CCARM 13322 was due mainly to a mutation in the QRDR of *gyrA*, while macrolide and tetracycline resistance was due to an A2075G mutation in the 23S rRNA and *tet*(O) (11–14). Since the other three genes—bla_{TEM}, integrase, and class I integron gene cassettes—were not amplified by PCR amplification, β-lactam and aminoglycoside resistance could not be characterized (15).

Even though no consensus has yet been reached on the definition and use of terms such as "multidrug resistant," "extremely drug resistant," "extensive, extensively, or extremely drug resistant (XDR)," and "pan-drug resistant" (16), results in this study suggest that *C. jejuni* CCARM 13322 is XDR. Until now, there has been no academic evidence that the *C. jejuni* CCARM 13322 clone was disseminated. To our knowledge, this is the first report of XDR *C. jejuni* recovered from a human case of diarrhea associated with international travel.

TABLE 3 Molecular characteristics of *C. jejuni* CCARM 13322

Test	Characteristic(s)
MLST	Clonal complex unassigned, ST5075
Detection of β-lactamase	pI 6.2 ESBL
QRDR mutations in *gyrA*	Silent nucleotide changes CAC→CAT, AGT→AGC, GCC→GCT and amino acid changes His-81→His, Ser-119→Ser, Ala-120→Ala
	Missense nucleotide change ACA→ATA and amino acid change Thr86→Ile
Gene mutation in peptidyl transferase region in domain V of 23S rRNA	A2075→G
tet(O) gene existence	Positive

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