

Short Communication

Antibiotic Resistance of *Campylobacter jejuni* and *C. coli* Isolated from Children with Diarrhea in Thailand and Japan

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SUMMARY: A total of 29 *Campylobacter jejuni* and *C. coli* strains were isolated from Thai and Japanese children with diarrhea using the Loop-mediated Isothermal Amplification method. The samples were evaluated for mutations in *gyrA* and *23S rRNA* in order to assess resistance against fluoroquinolones and macrolides, respectively. Among the isolated strains, 9 (8 *C. jejuni* and 1 *C. coli*) were from Thai children, and the other 20 (*C. jejuni*) were isolated from Japanese children. High fluoroquinolone resistance rates were observed in Thai (66.7%) and Japanese (90%) children. Macrolide resistance was not observed in Japanese children but was observed at a considerable rate of 12.5% of *C. jejuni* isolated in the Thai cohort. The results indicate that continuous monitoring of resistance of *Campylobacter* strains to fluoroquinolones and macrolides is definitely necessary.

Campylobacter jejuni and *C. coli* belong to the genus *Campylobacter* of the family *Campylobacteraceae*, and they are recognized among the most frequent causative factors of bacterial diarrhea worldwide, particularly in Southeast Asia (1–4). *Campylobacteriosis* is usually self-limiting; however, antibiotic therapy is required in the most severe infections. In addition, antibiotic therapy is often necessary for very young children, pregnant women, and elderly and immunocompromised individuals. Fluoroquinolones, such as ciprofloxacin, and macrolides, such as erythromycin and azithromycin, are the antibiotic agents of choice for campylobacteriosis. The increasing prevalence of *Campylobacter* isolates resistant to fluoroquinolones and macrolides is implicated in adverse patient outcomes, and is considered a serious public health problem. A previous study demonstrated that infection with either quinolone- or macrolide-resistant *Campylobacter* strains is associated with an increased risk of invasive illness or death, compared to infection with drug-susceptible *Campylobacter* strains (5). Macrolide and quinolone resistance in *Campylobacter* species is mainly a consequence of the use of antimicrobials in food-animal production. In *Campylobacter*, resistance to fluoroquinolones is mainly caused by chromosomal mutation in *gyrA*, which results in a Thr-

86-Ile substitution. This substitution is known to be responsible for high-level resistance to fluoroquinolone. The major mechanism conferring resistance to macrolides involves of an alteration of the target site (A2075G) in the peptidyl transferase region of *23S ribosomal RNA (rRNA)* genes. High-level resistant isolates carry a point mutation (A2075G) in domain V of *23S rRNA*, which is not observed in low-level resistant isolates (6). Currently, in addition to increasing widespread resistance of *Campylobacter* to fluoroquinolone, resistance to macrolides has been increasing in some countries (7,8). The aim of this study was to investigate the resistance of *C. jejuni* and *C. coli* isolated from Thai and Japanese children with diarrhea to fluoroquinolones and macrolides.

A total of 29 stool samples determined to be positive for either *C. jejuni* or *C. coli* using loop-mediated Isothermal Amplification and PCR methods, were analyzed to investigate the resistance to fluoroquinolones and macrolides by studying mutations in *gyrA* and *23S rRNA*, respectively. Of these 29 isolates, 9 (8 positive for *C. jejuni*, and 1 positive for *C. coli*) were collected from hospitalized children with diarrhea at Nakornping hospital, Chiang Mai, Thailand between January and October 2012 (9). The remaining 20 samples were collected at a pediatric outpatient clinic in Japan that was known to be positive for *C. jejuni* between July 2010 and June 2012 (10). The Institutional Review Boards of Nakornping hospital, Chiang Mai, Thailand and the pediatric outpatient clinic in Japan, as well as the Ethical Committee of the Nihon University School of Medicine approved this study (No. 22-15 and No. 25-13-0).

After DNA extraction, PCR was performed using the primers GZgyrA5 (5'-ATTTTGTAGCAAAGATTCTGAT-3') and GZgyrA6 (5'-CCATAAATTATTCACCTGT-3') to amplify 673 bp of *gyrA*, containing the quino-

Received December 24, 2014. Accepted April 13, 2015.
J-STAGE Advance Publication July 10, 2015.

DOI: 10.7883/yoken.JJID.2014.582

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lone resistance-determining region (QRDR) (11). For *C. coli*, the primer pair GZgyrACcoli3F (5'-TATGAGCGT TATTATCGGTC-3') and GZgyrACcoli4R (5'-GTCCA TCTACAAGCTCGTTA-3') was used to produce a 505 bp PCR product (12). To detect mutations in 23S *rRNA*, another PCR was performed using the specific primer pair F1-campy-23S (5'-AAGAGGATGTATAG GGTGTGACG-3') and R1-campy-23S (5'-AACGATTT CCAACCGTTCTG-3') to generate a 508 bp product (13). Amplification primers for *gyrA* and 23S *rRNA* were used as sequencing primers.

Sequence analysis of *gyrA* revealed that 23 of the 28 *C. jejuni* strains (82.1%) contained the Thr-86-Ile (ACA → ATA) *gyrA* mutation (Fig. 1). In addition, 5 of 8 (62.5%) Thai strains and 18 of 20 (90%) Japanese strains had the same mutation. The *C. coli* strain also contained the Thr-86-Ile (ACT → ATT) *gyrA* mutation (Fig. 1). The resistance rates of *C. jejuni* and *C. coli* to fluoroquinolones in Thailand and Japan were 66.7% and 90%, respectively.

Only 1 Thai *C. jejuni* strain (CMHN21/12) exhibited an A2075G mutation in 23S *rRNA*, which caused high-level resistance to macrolides (Fig. 2). This macrolide-resistant strain was also resistant to fluoroquinolones (Fig. 1). The resistance rate of *Campylobacter* was 11% in Thailand and 12.5% for Thai *C. jejuni* strains alone.

In this study, *C. jejuni* and *C. coli* strains isolated in Thailand and Japan demonstrated high fluoroquinolone resistance rates. Fluoroquinolone resistance rates of the Japanese strains were higher than that of a previous study conducted in Japan, which revealed that 37 of 55 (67.3%) *C. jejuni* isolates tested had the Thr-86-Ile

gyrA mutation (14).

Macrolide resistance was not observed in Japan in the current study, which correlates with previous findings showing that *C. jejuni* isolates were susceptible to clarithromycin and erythromycin (14). However, the resistance rate of *C. jejuni* (12.5%) observed in this study was greater than that previously reported for *C. jejuni* isolated from Thai children with diarrhea between 1991 and 2000 (from 0% to 6.3% in 1999) (8,15). This further confirms recent concerns of the increase in macrolide resistance of *C. jejuni* in Thailand. However, further investigations should be conducted using higher numbers of Thai samples.

In conclusion, the present study provides updated information on the fluoroquinolone and macrolide resistance rates of *C. jejuni* and *C. coli* isolated from children with diarrhea in Thailand and Japan. Our results demonstrate that erythromycin and other macrolides are still the first-choice treatment for severe cases of campylobacteriosis in Japan. However, in Thailand, macrolide resistance of *C. jejuni* should be taken into account, as the resistance rates have recently increased. Consequently, continuous monitoring of *Campylobacter* resistance to fluoroquinolones and macrolides is definitely necessary.

The nucleotide sequences of the *Campylobacter* strains described in this study have been deposited in the GenBank database under accession numbers: KP159387 to KP159415 (*gyrA*) and KP174777 to KP174805 (23S *rRNA*). The accession numbers for the reference strains are as follows: fluoroquinolone-resistant strains: DQ449657, DQ449658, AJ567826, AB894308,

	70	80	90	100
L04566-C.jejuni	TDFVKS	RIVGAVIGRY	HPHGH	TAVID ALVRMAQDFS MRYPST
DQ449657-C.jejuni	I
DQ449658-C.jejuni	I
AJ567826-C.jejuni	I
CMHN5/12-C.jejuni
CMHN64/12-C.jejuni
CMHN21/12-C.jejuni	I
CMHN1/12-C.jejuni	I
CMHN152/12-C.jejuni	I
CMHN54/12-C.jejuni	I
CMHN31/12-C.jejuni	I
CMHN15/12-C.jejuni
JP335-C.jejuni
JP556-C.jejuni	I
JP569-C.jejuni	I
JP183-C.jejuni	I
JP229-C.jejuni	I
JP334-C.jejuni	I
JP329-C.jejuni	I
JP579-C.jejuni	I
JP279-C.jejuni	I
JP537-C.jejuni	I
JP533-C.jejuni	I
JP526-C.jejuni	I
JP525-C.jejuni	I
JP333-C.jejuni	I
JP491-C.jejuni	I
JP284-C.jejuni	I
JP527-C.jejuni	I
JP561-C.jejuni
JP300-C.jejuni	I
JP558-C.jejuni	I
AF092101-C.coli	SAYK...	D...K.D
AB894314-C.coli	SAYK...	D...K.	ID
AB894308-C.coli	SAYK...	D...K.	ID
CMHN127/12-C.coli	SAYK...	D...K.	ID

Fig. 1. Alignment of the deduced amino acid sequences of the QRDR of the *gyrA* between the studied strains and reference *Campylobacter* strains. Position of mutation (Thr-86-Ile) is based on position of the complete *C. jejuni* UA580 *gyrA* (accession number L04566). Abbreviation for location: CMHN, Thailand; JP, Japan.

	2070	2080	2090	2100	2110
NCTC11168- <i>C. jejuni</i>	A	CGGAAGACC	CCGTGGACCT	TTACTACAGC	TTGACACTGC TAC
AX190985- <i>C. jejuni</i>
AY249920- <i>C. jejuni</i>	.	G.	.	.T.	.T
CMEN21/12- <i>C. jejuni</i>	.	G.	.	.	.T
CMEN64/12- <i>C. jejuni</i>T
CMEN1/12- <i>C. jejuni</i>
CMEN5/12- <i>C. jejuni</i>
CMEN15/12- <i>C. jejuni</i>
CMEN31/12- <i>C. jejuni</i>
CMEN54/12- <i>C. jejuni</i>
CMEN152/12- <i>C. jejuni</i>
JP183- <i>C. jejuni</i>
JP335- <i>C. jejuni</i>
JP229- <i>C. jejuni</i>
JP279- <i>C. jejuni</i>
JP284- <i>C. jejuni</i>
JP300- <i>C. jejuni</i>
JP329- <i>C. jejuni</i>
JP333- <i>C. jejuni</i>
JP334- <i>C. jejuni</i>
JP491- <i>C. jejuni</i>
JP525- <i>C. jejuni</i>
JP526- <i>C. jejuni</i>
JP527- <i>C. jejuni</i>
JP537- <i>C. jejuni</i>
JP558- <i>C. jejuni</i>
JP569- <i>C. jejuni</i>
JP579- <i>C. jejuni</i>
JP561- <i>C. jejuni</i>T
JP556- <i>C. jejuni</i>
JP533- <i>C. jejuni</i>
U09611- <i>C. coli</i>T
AY249918- <i>C. coli</i>	.	G.	.	.T.	.T
AY249921- <i>C. coli</i>	.	G.	.	.T.	.T
AY249922- <i>C. coli</i>	.	G.	.	.	.T
AY249923- <i>C. coli</i>	.	G.	.	.	.T
CMEN12712- <i>C. coli</i>

Fig. 2. Alignment of the sequences of the 23S *rRNA* associated with macrolide resistance between the studied strains and reference *Campylobacter* strains. Position of a point mutation (A2075G) is based on position of the reference *C. jejuni* isolate NCTC 1168 (accession number AL111168).

AB894314; fluoroquinolone-susceptible strains: L04566, AF092101; macrolide-resistant strains: AY249918 to AY249923; and macrolide-susceptible strains: AL111168 (strain NCTC11168), U09611, AY190985.

Acknowledgments This study was supported by Grants-in-Aid for Scientific Research under JSPS (Japan Society for the Promotion of Science) grant number 23406036 and Postdoctoral Fellowships (FY 2013–2015).

Conflict of interest None to declare.

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