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′-ATTTTTAGCAAAGATTCTGA-3′) and GZgyrA6 (5′-CCATAAATTATTCCACCTTGAAC-3′) to amplify 673 bp of gyrA, containing the quino-
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.
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 strain 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.

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Conflict of interest None to declare.

REFERENCES