Short Communication

Loop-Mediated Isothermal Amplification (LAMP) for Detection of Campylobacter jejuni and C. coli in Thai Children with Diarrhea

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SUMMARY: Campylobacter species are common causes of bacterial diarrhea, and Campylobacter jejuni and C. coli are known as the predominant causative agents in humans. Recent studies suggested that loop-mediated isothermal amplification (LAMP) is an efficient and practical tool for rapid detection of C. jejuni and C. coli in clinical samples. We used LAMP to screen 151 stool samples for Campylobacter; these samples were collected in 2012 from Thai children with diarrhea. The PCR method discriminated C. jejuni and C. coli among the detected Campylobacter strains; these species were subjected to sequencing of the hipO gene (in C. jejuni) or the ask gene (in C. coli). The results suggest that the prevalence of Campylobacter infection among Thai children with diarrhea is 8.6%, and C. jejuni is the most prevalent species.

The genus Campylobacter belongs to the family Campylobacteraceae and contains 18 species, 2 of which, C. jejuni and C. coli, frequently cause intestinal infections worldwide (1–3). Conventional culture testing is recognized as the gold standard for the diagnosis of Campylobacter diarrheal disease. This method, however, is complex and time-consuming and may fail to detect these microorganisms (4). Rapid and accurate diagnosis of Campylobacter infection in infants and children is necessary for timely (early) treatment. Recent studies suggested that loop-mediated isothermal amplification (LAMP) is an efficient and practical tool for the rapid detection of C. jejuni and C. coli in clinical samples and is more sensitive than the conventional culture method (4–7). The aim of this study was to test the LAMP method for detection of C. jejuni and C. coli in stool samples from children younger than 5 years of age who were admitted with diarrhea to Nakornping Hospital in Chiang Mai, Thailand, in 2012.

During the study period, from January to October 2012, we collected 151 stool samples. These samples were subjected to DNA extraction and then to LAMP for the detection of Campylobacter by means of the Loopamp DNA Amplification Kit (Eiken Chemical Co., Ltd., Tochigi, Japan). Additionally, the samples were tested for common viral pathogens including rotavirus, adenovirus, norovirus, sapovirus, astrovirus, Aichi virus, enterovirus, bocavirus, and human parechovirus by RT-PCR methods. Informed consent was obtained from the parents or legal guardians of all of the participants. Ethical approval was obtained from the Institutional Review Board of Nakornping Hospital and from the Ethics Committee of the Nihon University School of Medicine (decision numbers 22-15 and 25-13-0).

The samples that tested positive for Campylobacter according to LAMP were confirmed by PCR as infected either with C. jejuni or with C. coli. The primer pair hipO-F and hipO-R was used to amplify a 344-bp fragment of the hippurate (hipO) gene (specific for C. jejuni (8), and the primers CC18F and CC519R were used to amplify a 502-bp fragment of the aspartate kinase (ask) gene (specific for C. coli (9)). The samples that did not test positive for either C. jejuni or C. coli were subjected to semi-nested PCR with 2 newly developed forward primers described in our recent study (6), CJF2 and CCF2, in combination with the reverse primers HipO-R and CC519R to obtain a 169-bp amplicon and 277-bp amplicon that confirmed the presence of C. jejuni or C. coli, respectively. The amplicons from the genes hipO and ask were purified and sequenced (GenBank accession numbers KJ659822–KJ659832).

Of the 151 stool samples from Thai children with diarrhea, 13 (8.6%) were positive for Campylobacter. This prevalence rate is similar to our recent results from Japanese children with diarrhea (6) but lower than that in another study conducted in Thailand (18%) (1). The majority (10 of 13; 77%) of the Campylobacter-positive patients were admitted to the hospital in January and February. The mean age of the children with the Campylobacter infection was 13 months (range: 5–34), and most of the patients (8 of 13) were 12–14 months of age; this finding is in agreement with another study (10). Of the 13 Campylobacter-positive samples, 7 were co-
infected with another pathogen(s), such as group A rotavirus (3 samples), norovirus GII (2 samples), or enteric adenovirus (2 samples) (Table 1). The co-infection with Campylobacter and 1 of these diarrheal viruses was observed in 54% of the Campylobacter-positive cases; this level is higher than that reported in other studies (6,10).

The 344-bp fragment of the hipO gene was successfully amplified in 9 of the 13 Campylobacter-positive stool samples, indicating infection with C. jejuni. Three other samples were identified as C. jejuni (1 sample) and C. coli (2 samples) by semi-nested PCR. The remaining sample was negative for these 2 species when tested by the methods involving the above-mentioned primers but tested positive when evaluated by another PCR method involving the previously published primer pair DP1 and CJ1 generating a 300-bp amplicon from the 23S rRNA gene (11). After sequencing (GenBank accession No. KP399594) and a BLAST search, this Campylobacter species was identified as C. coli, with an identity of 98% with the reference strain CCARM 13271 (Table 1). The false negative result might be caused by a mismatch between the primers CC18F and CC519R and the genome of this C. coli strain.

In conclusion, according to the LAMP method, the prevalence of Campylobacter infection among hospitalized Thai children with diarrhea is 8.6%. C. jejuni seems to be the most prevalent species. Co-infection with group A rotavirus, enteric adenovirus, or norovirus GII was detected in 54% of the Campylobacter-positive cases.

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Conflict of interest None to declare.

REFERENCES