Short Communication

High Prevalence of *Campylobacter ureolyticus* in Stool Specimens of Children with Diarrhea in Japan

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**SUMMARY:** *Campylobacter ureolyticus* has been considered as a potentially pathogenic bacterium. In this study, a total of 586 stool samples were collected from 0–12-year-old children with diarrhea between November 2013 and April 2015 and examined with microbiological tests in the hospital for the diagnosis of common enteric pathogens including *C. jejuni* and *C. coli*. Then in our laboratory, these samples were analyzed by 16S rRNA sequence-based *Campylobacter* genus-specific PCR (C16S PCR); 283 (48.3%) samples showed positive results with this PCR assay. Furthermore, *C. ureolyticus* was screened in these 283 samples by PCR assay, which can detect this species specifically. Surprisingly, *C. ureolyticus* was detected in 147 of the 283 C16S PCR-positive diarrheal stool samples (51.9%), which is much higher than the prevalence of *C. jejuni* and *C. coli* (15.5%), and 96 samples out of 147 were negative for any of the other enteric pathogens tested in the hospital; namely, *C. ureolyticus* was detected as a single pathogen in 96 samples. This finding suggests that *C. ureolyticus* may be a pathogen associated with diarrhea in children in Japan. To the best of our knowledge, this is the first report in which *C. ureolyticus* was detected among Japanese children with diarrhea.

Among 26 *Campylobacter* species (spp.) *C. jejuni* and *C. coli* are the most frequently isolated species from human diarrheal patients (1). Recently, by using non-selective media and species-specific gene detection methods, other *Campylobacter* spp. such as *C. concisus* and *C. upsaliensis*, have been increasingly detected and isolated from diarrheal patients (2,3). Additionally, *C. ureolyticus* has been suggested to be an emerging pathogen causing gastroenteritis in humans (4,5). Bullman et al. (4) analyzed 7,194 stool samples from diarrheal patients in Ireland to detect *Campylobacter* and found that the species-specific PCR could detect *C. ureolyticus* from 83 (22%) out of 373 stool samples, which were positive for *Campylobacter* spp. As expected, *C. jejuni* (246 samples, 66%) was the most predominant species. Since the study was carried out with patients in a limited area, more epidemiological surveys in different areas are needed to understand whether *C. ureolyticus* is a possible emerging enteric pathogen worldwide. In this study, the prevalence of *C. ureolyticus* was examined in stool specimens of children with diarrhea in which *Campylobacter* genus-specific PCR products were obtained in Japan. Simultaneously, all stool samples were subjected to microbiological and molecular diagnosis for the presence of common enteric pathogens.

A total of 586 rectal swabs were collected from 0–12-year-old children with diarrhea who visited the Department of Pediatrics, Mizushima Central Hospital, Okayama, Japan between November 2013 and April 2015. Microbiological tests were carried out with culture methods and PCR was performed for enteropathogenic bacteria including *Campylobacter* and *Salmonella*, and immunochromatography was performed for Norovirus, Rotavirus, and Adenovirus in the hospital for the diagnosis of common enteric pathogens. The results of microbiological tests are summarized in Table 1.

<table>
<thead>
<tr>
<th>Pathogens detected¹⁾</th>
<th>Number of positive specimens by the C16S PCR²⁾</th>
<th>Number of positive specimens by the <em>C. ureolyticus</em> PCR²⁾</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. jejuni/C. coli</em></td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td><em>C. jejuni/C. coli</em> and Norovirus</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>Norovirus</td>
<td>41</td>
<td>14</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>39</td>
<td>22</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Others⁴⁾</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>167</td>
<td>97</td>
</tr>
<tr>
<td>ND⁶⁾</td>
<td>419</td>
<td>186</td>
</tr>
<tr>
<td>Total</td>
<td>586</td>
<td>283</td>
</tr>
</tbody>
</table>

¹⁾ Pathogens were detected and isolated at Mizushima Central Hospital.
²⁾ No. of positive specimens by the C16S PCR.
⁴⁾ No. of positive specimens by the *C. ureolyticus*-specific PCR.
⁵⁾ Others including *Influenza* spp., *Versinia* spp., *Klebsiella oxytoca*, both *Acrononas* spp. and *Norovirus*, and *Streptococcus* spp.
⁶⁾ No. of total samples which other enteric pathogens were detected in hospital.


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Simultaneously, the rectal swabs kept in Cary-Blair medium (Becton Dickinson and Company, Franklin Lakes, NJ, USA) were immediately transferred to Osaka Prefecture University at an ambient temperature. Then rectal swabs were suspended in 500 µL phosphate-buffered saline and a DNA template was prepared by the boiling method to perform cdt gene-based multiplex PCR for detecting C. jejuni, C. coli, and C. fetus (6), and C16S PCR using a Campylobacter genus-specific primer set of C16S-F3 (5′-GGAGGATGACACTTTTCG-3′) and C16S-R5 (5′-CGTATTACTAGCGATTCGG-3′), which were designed from conserved regions in 16S rRNA genes of 26 Campylobacter spp. The PCR mixture contained 0.5 µM of each primer, 1 µL of the DNA template, 0.2 mM of dNTP mixture, rTaq DNA polymerase buffer, and 1.0 U of rTaq DNA polymerase (Takara Bio Inc., Shiga, Japan) in a 20 µL reaction volume. PCR was performed in the Takara PCR Thermal Cycler (Takara Bio Inc.) or Applied Biosystems GeneAmp PCR 9700 (Life Technologies Co., Carlsbad, CA, USA). PCR products were analyzed by electrophoresis using 2% PrimeGel™ Agarose LE gel (Takara Bio Inc.) and bands were visualized by UV light after staining with ethidium bromide (1 µg/mL). Images were captured on a Bio-Rad Chemi Doc system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). As shown in Table 1, of 586 samples, 283 (48.3%) were positive for the C16S PCR. Interestingly, the Campylobacter genus-specific PCR amplicon was obtained from not only the 41 samples in which C. jejuni or C. coli was detected at the hospital but also the 242 samples, which were negative for Campylobacter using routine culture methods. This finding strongly suggests that the 242 stool samples might contain Campylobacter spp. other than C. jejuni and C. coli.

To identify Campylobacter spp. in the C16S PCR-positive samples, we first sequenced the C16S PCR amplicons from 8 randomly selected samples in which no enteric pathogen was detected by microbiological tests. Sequencing results indicated the presence of C. hominis, which has been proposed as a commensal bacterium in humans (7), in 5 samples and C. ureolyticus in 3 samples. Subsequently, we examined the prevalence of C. ureolyticus in the 283 samples that were positive for C16S PCR (Table 1). The corresponding DNA template was subjected to PCR using the C. ureolyticus spp.-specific PCR primer set developed by Bullman et al. (4). Surprisingly, C. ureolyticus was detected in 147 out of the 283 C16S PCR-positive samples (51.9%), which is much higher than the detection rate of C. jejuni and C. coli (15.5%). It is notable to emphasize that the prevalence rate of C. ureolyticus in stool samples in this study is much higher than that in southern Ireland (22%) (4). To the best of our knowledge, this is the first report of a study wherein C. ureolyticus was detected among Japanese children with diarrhea, and in some of whom, it was detected as a single pathogen.

In conclusion, C. ureolyticus may be associated with diarrhea in children in Japan. Further surveillance of enteric pathogens, particularly C. ureolyticus, in children with diarrhea is required to understand its importance as an enteric pathogen by using both culture and genetic methods.

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Conflict of interest None to declare.

REFERENCES
5. Collado L, Gutierrez M, Gonzalez M, et al. Assessment of the prev-
C. ureolyticus May Be a Diarrheal Pathogen


