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(日本語表記)

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齊木 大、前田 雅子、浅山 睦子、鈴木 淳、貞升 健志
SUMMARY (176 words)

*Campylobacter jejuni* is a major foodborne pathogen causing enteritis in humans and is also known as an antecedent infectious factor for Guillain-Barré syndrome (GBS). The onset of GBS after *C. jejuni* infection results from molecular mimicry between human neuronal ganglioside and *C. jejuni* lipooligosaccharide (LOS). *C. jejuni* HS:19 has been previously reported to be isolated from GBS cases more frequently than other serotypes in Japan. Therefore, in this study, we performed molecular analysis of 88 HS:19 isolates from GBS cases, sporadic diarrheal patients, and poultry meats using multi-locus sequence typing and LOS class analysis. As a result, 87 of the 88 HS:19 isolates were typed as ST22 / CC22 and LOS class A1, while one was typed as ST1947 / CC22 and LOS class A1. Furthermore, the analysis of other 331 isolates from sporadic enteritis cases shows that only 34 (10.3%) were typed as LOS class A, including HS:19 (25 isolates), HS:2 (8 isolates), and HS:4c (1 isolate). In conclusion, *C. jejuni* HS:19 had high clonality, regardless of its origin, over other capsule types in Japan.
Short Communication (1,200 words)

*Campylobacter jejuni* is a globally widespread and major foodborne enteritis agent in humans and is frequently transmitted from chicken meat, unpasteurized milk, and contaminated water, among others (1). In recent years, food poisoning caused by *C. jejuni* has been occurring frequently in Japan, especially because of the consumption of raw or insufficiently heated poultry meat (2). In addition, after *C. jejuni* enteritis, the onset of Guillain-Barré syndrome (GBS) with bilateral flaccid motor paralysis rarely occurs (3).

*C. jejuni* has been reported to be one of the causative agents for the GBS, which accounted for approximately 32% of the GBS cases reported (4). Nonetheless, epidemiological insights into GBS are unclear (5). Molecular mimicry between human neuronal gangliosides (e.g., GM1) and the lipooligosaccharide (LOS) that constitutes the outer membrane of *C. jejuni* has been proposed as a GBS onset factor (6). In particular, some reports have indicated structural similarities between *C. jejuni*, which expresses sialylated LOS (class A, B, C), and sugar chains such as GM1 (7-9). In addition, among 102 isolates of *C. jejuni* from patients with GBS in Japan, it was reported that the HS:19 strain accounted for 67 isolates (65.7%) (10). Therefore, to study the molecular characteristics of *C. jejuni* HS:19 and the distribution of sialylated LOS class A, B, and
C, we analyzed multi-locus sequence typing (MLST) and LOS class of *C. jejuni* HS:19 isolated from patients with GBS, sporadic diarrheal patients, and poultry meat in Japan. Furthermore, to study the general distribution of sialylated LOS class A, B, C—which have an important role as GBS onset factors—in *C. jejuni* strains other than HS:19, we analyzed the LOS class and its association with *C. jejuni* capsule types isolated from domestic diarrheal patients three years before this study.

All study procedures were reviewed and approved by the Institutional Review Board of Tokyo Metropolitan Institute of Public Health (TMIPH) according to the Declaration of Helsinki 2013 (Permit No. 27KenKenKen-511, 30KenKenKen-782). For MLST and LOS class analysis, we used 88 isolates of *C. jejuni* HS:19 including 30 isolates from patients with GBS (10), 46 from sporadic diarrheal patients and 12 from poultry meat collected from 1990 to 2015 in Japan. Regarding the relationship between LOS class and *C. jejuni*, capsule types 331 other isolates from sporadic diarrheal patients in Tokyo from 2017 to 2019 were used (Table 1). For capsule-typing and LOS class analysis, a small number of *C. jejuni* colonies grown on Brucella agar supplemented with 5% horse defibrinated blood at 37 °C for 24 h under microaerobic conditions (5% O₂, 10% CO₂, 85% N₂) were taken. They were suspended in 100 μL of sterile distilled water containing 20 μL of 10 mM NaOH and heated at 100 °C for 10 min, followed by centrifugation at 13,000 x g for 3
The supernatant obtained was used as template DNA. Furthermore, for MLST analysis, *C. jejuni* strains were cultured with shaking in brain heart infusion broth at 37 °C for 24 h under microaerobic conditions. Subsequently, genomic DNA was extracted from the cultured medium using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany).

Capsule-typing was performed using 37 pairs of primers reported previously (11) that can specifically detect serotype-related genes via the Penner’s method (12). Multiplex polymerase chain reaction (PCR) was performed with 37 pairs of primers divided into four PCR tubes using 10 μL of KOD SYBR qPCR Mix (TOYOBO, Osaka, Japan), 0.5 μM forward and reverse primers, 2 μL of template DNA, and the total volume of reaction per tube was adjusted to 20 μL with sterile distilled water. As for the PCR conditions, after 2 min of initial denaturation at 98 °C, 20 cycles of 98 °C for 10 s, 50 °C for 10 s, and 68 °C for 30 s were carried out. The isolates that could not be capsule-typed were classified as Untypable (UT). We designed new primers for the real-time PCR to classify LOS A, B, and C after referring to a previous report (9). The reaction conditions for the real time PCR were the same as those described above for the capsule-typing method. The types other than LOS class A, B, and C were determined as ‘others’. MLST analysis was conducted as per the method published on the *Campylobacter* MLST Home Page (http://pubmlst.org/campylobacter/).
We focused on the epidemiological fact that HS:19 was isolated from GBS patients more than other serotypes in Japan (10). Of the 88 isolates, 87 were classified as ST22/CC22 and LOS A1; one isolate was typed as ST1947/CC22 and LOS A1. However, the difference between ST22 and ST1947 was only a single base mutation in \textit{glnA} while both belonged to CC 22. Both of these sequence types (ST) were closely related (Table 2). As for the prevalence of capsule types, HS:2 (72/331), HS:4c (65/331), HS:23c (29/331), HS:19 (25/331), and HS:8, 17 (24/331) were frequently detected (Table 3). Among the 331 isolates derived from diarrheal patients, 213 (64%) could be classified as LOS class A, B, or C. Regarding the prevalence of LOS classes, that of LOS class A1 was 33/331, LOS class A2 was 1/331, LOS class B1 was 40/331, LOS class B2 was 91/331, LOS class C was 48/331, and ‘others’ was 118/331 (Table 3). Especially, LOS class A was detected only in HS:19, HS:2, and HS:4c. Moreover, LOS class B was detected in HS:2 and HS:4c, LOS class C in HS:1, 44, and HS:8, 17 was detected in relatively higher percentages than other capsule types (Table 3). In this study, we found that 87 of 88 \textit{C. jejuni} HS:19 isolates belonged to ST22/CC22 and LOS class A1 regardless of their origins. Isolates of \textit{C. jejuni} belonging to the same capsule type often identified with different ST and LOS classes (13). It was suggested that the clonality of HS:19 was very high compared to other capsule types (8). Except for HS:19, a few \textit{C. jejuni} isolates were
found to be typed as LOS class A; LOS class A1 was found in only 8 isolates of HS:2 and LOS class A2 in only one isolate of HS:4c. In patients with GBS who had antecedents of *C. jejuni* infection, the capsule types HS:1/44, HS:2, HS:4c, HS:19, HS:23c, and HS:41 were reported to be classified under LOS class A, B, and C (8,10). In previous studies, GBS cases caused by antecedent *C. jejuni* HS:41 infection were reported in South Africa (14); moreover, it was reported that capsule type HS:23c, HS:19, and HS:41 were most prevalently associated with GBS in Bangladesh (8). Regarding MLST analysis, there were a few ST22 *C. jejuni* type in France (13), different from this study in Japan. These findings suggested that *C. jejuni* capsule types associated with GBS vary geographically or are dependent on some host factors, leading to GBS onset (15). In conclusion, our study clarifies that *C. jejuni* HS:19 types as ST22/CC 22 and LOS class A1, regardless of their origins. Moreover, LOS class A was hardly detected in other capsule types. Furthermore, the clonality of HS:19 is higher than those of other capsule types.

**Acknowledgments**

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Conflict of interest  None to declare.

REFERENCES

1. European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC). The European Union one health 2018 zoonoses report. EFSA J. 2019;17:5926.


Table 1. *Campylobacter jejuni* isolates used for MLST and LOS class analyses.

<table>
<thead>
<tr>
<th>Capsule type of isolates</th>
<th>MLST and LOS class analysis</th>
<th>LOS class analysis only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GBS</td>
<td>Enteritis</td>
</tr>
<tr>
<td>HS:19</td>
<td>30</td>
<td>46</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

HS, Heat-stable; MLST, multi-locus sequence typing; LOS, lipooligosaccharide; GBS, Guillain-Barré syndrome
Table 2. MLST of *Campylobacter jejuni* HS:19 isolates typed as LOS class A

<table>
<thead>
<tr>
<th>Origin</th>
<th>Number of isolates</th>
<th>MLST (ST / CC )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ST22 / CC22</td>
</tr>
<tr>
<td>Patients with GBS</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Sporadic diarrheal patients</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Poultry meat</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>87</td>
</tr>
</tbody>
</table>

ST, sequence type; CC, Clonal complex; MLST, multi-locus sequence typing; LOS, lipooligosaccharide; GBS, Guillain-Barré syndrome
Table 3. Association between capsule type and LOS class in *Campylobacter jejuni* isolates from sporadic diarrheal patients

<table>
<thead>
<tr>
<th>LOS class</th>
<th>1,44</th>
<th>2</th>
<th>3</th>
<th>4c</th>
<th>6,7</th>
<th>8,17</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>19</th>
<th>21</th>
<th>23c</th>
<th>29</th>
<th>31</th>
<th>37</th>
<th>42</th>
<th>45</th>
<th>55</th>
<th>UT</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>25</td>
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<td></td>
<td></td>
<td>33  (10.0)</td>
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<tr>
<td>A2</td>
<td>1</td>
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<td></td>
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<td></td>
<td></td>
<td>1   (0.3)</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>29</td>
<td>1</td>
<td>4</td>
<td>1</td>
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<td>40  (12.1)</td>
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<tr>
<td>B2</td>
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<td>C</td>
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<td>48  (14.5)</td>
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<td>9</td>
<td>7</td>
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<td>3</td>
<td>1</td>
<td>6</td>
<td>3</td>
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<td>1</td>
<td>5</td>
<td>23</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>118 (35.6)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>72</td>
<td>20</td>
<td>65</td>
<td>7</td>
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<td>6</td>
<td>7</td>
<td>4</td>
<td>6</td>
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<td>5</td>
<td>29</td>
<td>1</td>
<td>2</td>
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<td>118 (35.6)</td>
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