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Epidemiology and Molecular Analysis of Group A Streptococci from Patients Involved in Food-Borne Disease Outbreaks in Japan between 1996 and 2003

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Group A streptococcus (GAS) is a pathogen responsible for human infection through various modes. Acute pharyngitis caused by GAS is common among children. Recently, various molecular techniques have been applied to the epidemiological investigation of GAS isolates. For example, emm typing, which involves the sequence analysis of the 5’ end of the emm gene that encodes the M protein, has been widely used to characterize GAS isolates because of its high discrimination power and versatility. The Centers for Disease Control and Prevention (CDC) maintains a database (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.html) containing >150 emm types for GAS. Multilocus sequence typing (MLST) is also a highly discriminatory and unambiguous method of characterizing bacterial isolates. MLST is based on the sequence variation of the internal fragments of housekeeping genes. The different sequences at each locus are assigned different allele numbers, and each unique profile is assigned a sequence type (ST). Enright et al. (1) developed a method used on S. pyogenes, and a public database (http://spyogenes.mlst.net/) is maintained.

In Japan, there have been frequent reports of large food-borne outbreaks of streptococcal pharyngitis in recent years. Since the late 1990s, seven food-borne outbreaks caused by GAS have been reported (Table 1), as opposed to only two cases up to and including the early 1990s (2,3). According to a review on food-borne streptococcal pharyngitis (4), the main cause of this infection is the poor handling and preservation of cold salads that generally contain eggs; further, epidemics tend to occur in warm months. We found this to be true of the recent food-borne streptococcal epidemics in Japan as well—egg was the most common ingredient, and all epidemics took place in warm months, i.e., between May and September. Therefore, to prevent food-borne streptococcal pharyngitis as well as other food-borne diseases, food handlers should pay great attention to personal hygiene when handling food.

We performed T serotyping, emm typing, and MLST for 16 GAS isolates from seven food-borne outbreaks in Japan during 1996-2003 (Table 1). Five T/emm types were found: T1/emm1, T22/emm22, T28/emm28, TB3264/emm68, and

<table>
<thead>
<tr>
<th>Date</th>
<th>Place (Prefecture)</th>
<th>Setting</th>
<th>No. of patients</th>
<th>Vehicle</th>
<th>No. of isolates</th>
<th>T type</th>
<th>emm type</th>
<th>ST</th>
<th>gks</th>
<th>ggr</th>
<th>mutL</th>
<th>mutS</th>
<th>recP</th>
<th>spt</th>
<th>yqiL</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1996.5</td>
<td>Aichi</td>
<td>Sports meeting</td>
<td>244</td>
<td>Catering lunch, including boiled eggs, boiled fish paste, fried chicken, and wakame-gohan</td>
<td>3</td>
<td>T1</td>
<td>1</td>
<td>28</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1997.5</td>
<td>Fukuoka</td>
<td>Police guard on international conference</td>
<td>943</td>
<td>Catering lunch, including rolled omelet</td>
<td>1</td>
<td>TB3264</td>
<td>104</td>
<td>405</td>
<td>1)</td>
<td>25</td>
<td>37</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>56</td>
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<td>1997.7</td>
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<td>Beer festival</td>
<td>77</td>
<td>Unknown</td>
<td>3</td>
<td>T22</td>
<td>22</td>
<td>45</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>10</td>
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<tr>
<td>1998.8</td>
<td>Ibaraki</td>
<td>Softball tournament</td>
<td>342</td>
<td>Catering lunch, including thick omelet</td>
<td>3</td>
<td>T22</td>
<td>22</td>
<td>45</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>11</td>
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<tr>
<td>1998.9</td>
<td>Kumamoto</td>
<td>Convention of a labor union</td>
<td>254</td>
<td>Sandwich, including egg salad</td>
<td>4</td>
<td>T28</td>
<td>28</td>
<td>52</td>
<td>11</td>
<td>6</td>
<td>14</td>
<td>5</td>
<td>9</td>
<td>17</td>
<td>19</td>
<td>12</td>
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<td>2003.9</td>
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<td>School trip</td>
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<td>Unknown</td>
<td>1</td>
<td>T28</td>
<td>28</td>
<td>52</td>
<td>11</td>
<td>6</td>
<td>14</td>
<td>5</td>
<td>9</td>
<td>17</td>
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<tr>
<td>2003.9</td>
<td>Chiba</td>
<td>Funeral</td>
<td>67</td>
<td>Catering lunch</td>
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<td>TB3264</td>
<td>68</td>
<td>247</td>
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<td>1</td>
<td>7</td>
<td>2</td>
<td>8</td>
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</table>

1) New designation by multilocus sequence typing (MLST).
ST, sequence type.

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TB3264/emm104. Types T22/emm22 and T28/emm28 matched the types from two independent food-borne outbreaks. In our previous study (5), T1/emm1 and T28/emm28 were dominant among throat and skin isolates, respectively; but T22/emm22, TB3264/emm68, and TB3264/emm104 were not common among throat and skin isolates. Thus, these results suggest that the presumed source of food contamination was not restricted to the food handler who had pharyngitis or hand wounds. The raw materials might have been contaminated by the pathogen before cooking. Close correlations between emm type and MLST were found among the isolates obtained over a long period and/or from different places in Japan. By using emm typing and MLST, a similar concordance has been noted in previous studies (1,6,7). Finally, our data generated one new ST (ST405) and provided useful comparative data for future studies on the epidemiological investigation of food-borne streptococcal pharyngitis.

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REFERENCES