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Phylogenetic Analysis of *Salmonella* Enteritidis Isolates from Food Poisonings Using Pulsed-Field Gel Electrophoresis over the Period of June 1997 to December 2000 in Hyogo Prefecture

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Since 1989, *Salmonella* serovar Enteritidis has become the most prevalent among the serotypes of *Salmonella* food poisonings in Japan (1,2). We previously reported the genetic variations of the 21 isolates obtained at the food poisonings that occurred from 1997-1999 in Hyogo Prefecture (3) together with detailed analyses of three outbreak cases (4,5). In that study, pulsed-field gel electrophoresis (PFGE) was used to identify six different genotypes, A, A’, B, C, D, and E. Fourteen strains belonged to genotype A and three to subtype A’ (3). Genotype A was further classified into two subtypes, A-a and A-b, according to the size of the largest band >630 kb; the band in A-a moved more slowly than that in A-b. There were eight A-a subtypes and six A-b subtypes (Figs. 1 and 2). Others were classified as genotypes B, C, D, and E (3).

We carried out a similar genotypic analysis of eight new isolates from seven food poisoning cases in Hyogo Prefecture...

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in 2000 (cases 22-28 in Table). Three phage types (PTs) were identified: PT 1, PT 14b, and PT 47. The strains were tested for the sensitivity to 12 drugs, ampicillin (ABPC), cefotaxime (CTX), kanamycin (KM), gentamicin (GM), streptomycin (SM), tetracycline (TC), trimethoprim (TMP), ciprofloxacin (CPFX), fosfomycin (FOM), chloramphenicol (CP), sulfamethoxazole-trimethoprim (ST), and nalidixic acid (NA), by Sensi Disk (Nippon Becton Dickinson Co., Ltd., Tokyo) (6). All, except two SM-resistant isolates (cases 23 and 24), were sensitive to the 12 antibiotics.

These eight isolates were examined by PFGE using a gene path typing system (Program No.2; Nippon Bio-Rad, Tokyo) (4), and the patterns were compared with those of the previous 21 isolates in 1997-1999. PFGE of BlnI chromosomal DNA digests (Fig. 1) revealed the presence of five genotype A (3) (A-a; cases 22, 25, 27, 28, and A-b; case 26), and two non-descript (3) (cases 23 and 24) (Fig. 1A). No correlation was observed between PFGE patterns and phage types (Table) as in our previous observation (3). Figure 1B shows representative PFGE patterns observed among 28 Salmonella from food poisonings in Hyogo Prefecture in 1997-2000.

A dendrogram obtained by molecular analysis software (Finger Printing Plus; Bio-Rad, Hercules, Calif., USA) for the isolates in 1997-2000 is shown in Figure 2. Two large clusters were observed, each consisting of several subclusters. Various genotypically different characteristics including phage type (1-5, 7) and PFGE pattern (2-5, 7) were observed in studies of a large-scale epidemic in Japan (1,2) as well as

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**Table. Food poisoning outbreaks of Salmonella Enteritidis in 2000 in Hyogo Prefecture and causative strains**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Date</th>
<th>Occasion</th>
<th>Exposed</th>
<th>Patients</th>
<th>Causative strain used</th>
<th>Strain*</th>
<th>Phage type</th>
<th>Drug resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Apr. 2000</td>
<td>Lunch served for old men by a volunteer group in a town</td>
<td>48</td>
<td>20</td>
<td>Hyogo-SE160</td>
<td>Hyogo-SE172</td>
<td>47</td>
<td>None</td>
</tr>
<tr>
<td>23</td>
<td>Aug. 2000</td>
<td>Chinese dishes at a hotel in Osaka City (Osaka Prefecture)**</td>
<td>4</td>
<td>3</td>
<td>Hyogo-SE177</td>
<td>1</td>
<td>SM</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Aug. 2000</td>
<td>Funeral meal at an assembly hall in Osaka City (Osaka Prefecture)**</td>
<td>318</td>
<td>140</td>
<td>Hyogo-SE178</td>
<td>1</td>
<td>SM</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Aug. 2000</td>
<td>Sushi at a sushi-bar in Kobe City (Osaka Prefecture)**</td>
<td>Unknown</td>
<td>102</td>
<td>Hyogo-SE179</td>
<td>14 b</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Oct. 2000</td>
<td>Wedding reception at a restaurant in Kyoto City (Kyoto Prefecture)**</td>
<td>69</td>
<td>29</td>
<td>Hyogo-SE187</td>
<td>1</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Nov. 2000</td>
<td>Egg from a supermarket at home in Kakogawa City (Osaka Prefecture)</td>
<td>2</td>
<td>2</td>
<td>Hyogo-SE190</td>
<td>47</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Dec. 2000</td>
<td>Raw-liver and -beef at a restaurant in Akoh City (Kyoto Prefecture)</td>
<td>3</td>
<td>3</td>
<td>Hyogo-SE193</td>
<td>47</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

* The strains used were all human origin except Hyogo-SE172, an isolate from the causative food.
** A part of the patients related to the poisonings were residents in Hyogo Prefecture.
in our previous (3-5) and next studies (7).

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REFERENCES


