

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

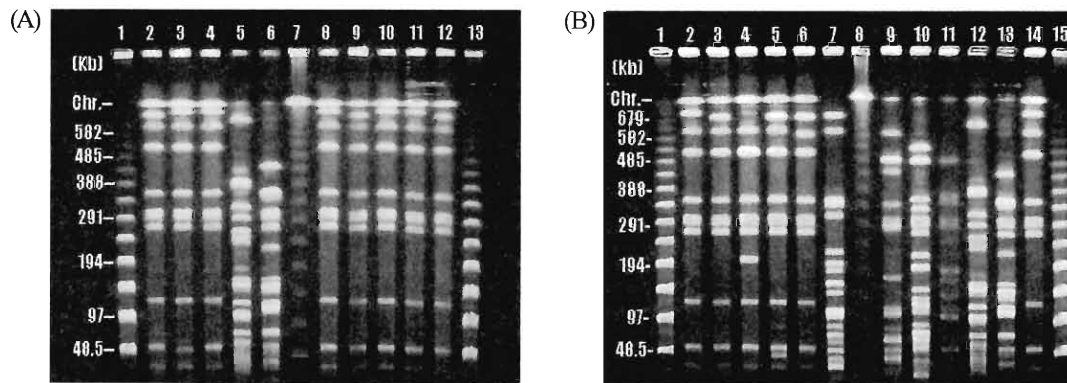


Fig. 1. PFGE patterns of *BlnI*-digests of chromosomal DNA of *Salmonella* Enteritidis isolates from 28 cases of food poisoning outbreaks. (A) *Salmonella* Enteritidis from food poisoning outbreaks (cases 22-28) in Hyogo Prefecture in 2000.

Lanes 1, 7, and 13:  $\lambda$  DNA ladder. Lane 3: Hyogo-SE160 (genotype A-a) from a patient in case 22. Lane 4: Hyogo-SE172 (A-a) from the causative food, omelet, in the same case. Lane 5: Hyogo-SE177 (nondescript) in case 23. Lane 6: Hyogo-SE178 (nondescript) in case 24. Lane 8: Hyogo-SE179 (A-a) in case 25. Lane 9: Hyogo-SE187 (A-b) in case 26. Lane 10: Hyogo-SE190 (A-a) in case 27. Lane 11: Hyogo-SE193 (A-a) in case 28. Lanes 2 and 12: Hyogo-SE186 (A-b) and Hyogo-SE189 (A-b): two isolates from sporadic cases 53 and 54 in 2000 as ref. 7. Chr: chromosome.

(B) Representative PFGE patterns of *Salmonella* Enteritidis isolates in food poisoning outbreaks in Hyogo Prefecture in 1997-2000.

Lanes 1, 8, and 15:  $\lambda$  DNA ladder. Lane 2: Hyogo-SE017 (genotype A-a) in case 6. Lane 3: Nishinomiya-01 (A-b) in case 2. Lane 4: Hyogo-SE103 (A') in case 14. Lane 5: Hyogo-SE008 (A') in case 5. Lane 6: Hyogo-SE127 (A') in case 17. Lane 7: Hyogo-SE065 (B) in case 10. Lane 9: Hyogo-SE151 (C) in case 20. Lane 10: Kakogawa-A03 (D) in case 1. Lane 11: Sumoto-21 (E) in case 3. Lane 12: Hyogo-SE177 (nondescript) in case 23. Lane 13: Hyogo-SE178 (nondescript) in case 24. Lane 14: Hyogo-SE193 (A-a) in case 28. Chr.: chromosome.

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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-descriptors (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

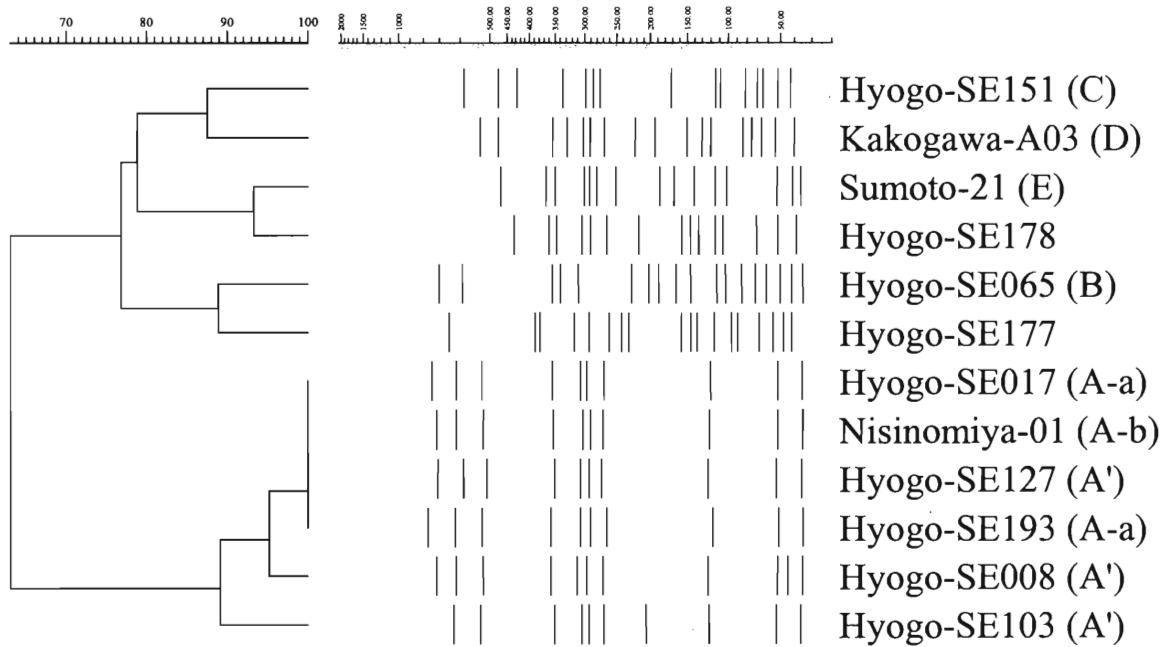


Fig. 2. Cluster analysis of PFGE patterns of *Salmonella* Enteritidis. Bands below 48.5 kb were omitted from the analysis. Genotypes are indicated in parentheses.

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

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

\* 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.

Jpn. J. Infect. Dis., 54, 2001

in our previous (3-5) and next studies (7).

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