

## Laboratory and Epidemiology Communications

# Phylogenetic Analysis of Genotypic Variations of *Salmonella* Enteritidis Isolates from Sporadic Infections Using Pulsed-Field Gel Electrophoresis from December 1996 to November 2000 in Hyogo Prefecture

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Communicated by Shinsaku Imashuku

(Accepted April 5, 2001)

Since 1989, *Salmonella* serovar Enteritidis has become the most prevalent among the *Salmonella* serotypes in Japan (1,2). In the previous (3-5) and preceding papers (6) in which were reported 28 food poisoning outbreaks caused by *Salmonella* Enteritidis from June 1997 to December 2000 in Hyogo Prefecture, we demonstrated, using pulsed-field gel electrophoresis (PFGE), a clear phylogenetic relationship among the causative strains for these food poisonings (6). We report here the genotypic variations and phylogenetic analysis of the total 55 isolates, including 50 from human feces in sporadic infection cases, two from samples from chickens, and three from chicken egg samples (Table).

Twenty-four of the 55 isolates were tested for phage types (PTs), the types being PT1, PT4, PT6, PT6a, PT9b, RDNC (Reaction Does Not Conform), and UT (Untypable). The remaining 31 isolates were not tested (Table). All of the 55

isolates were tested for 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) as reported in a previous paper (7). The majority (31 strains) were sensitive to the drugs, while the remaining 24 strains were resistant to SM, ABPC, TC, and/or GM (Table).

All of the 55 isolates were examined for PFGE patterns using a gene path typing system (Program No. 2; Nippon Bio-Rad, Tokyo) in a manner reported previously (3), except that lysozomal lysates were further treated with 1% sodium laurylsulfate for 1 h at 50°C for complete lysis. The typical PFGE patterns are shown in Figures 1 A and 1 B and summarized in Table. The PFGE patterns obtained with *Bln1*-digested chromosomal DNAs showed 15 different patterns. A dominant

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Table. List of *Salmonella* Enteritidis strains of sporadic occurrences over the past 4 years from December 1996 to November 2000<sup>1)</sup>

Case No.	Strain	Source (sex, age)	Date of sampling (feces/foods)	Phage type	Drug resistance <sup>2)</sup>	PFGE pattern: same as
1	Tatsuno-03	Human (F, 20)	Sept. 1997	Not tested	SM	Case 1
2	'96-S. 070	Human (F, 0-11)	Dec. 1996	Not tested	SM	Case 2
3	'96-S. 076	Human (M, 6)	Jan. 1997	Not tested	None	Case 3
4	'96-S. 090	Human (F, 2)	Mar. 1997	Not tested	None	Case 3
5	'97-S. 001	Human (M, 1)	Mar. 1997	Not tested	SM	Case 5
6	'97-S. 033	Human (F, 9)	Aug. 1997	Not tested	SM	Case 1
7	'97-S. 039	Human (M, 9)	Aug. 1997	Not tested	SM	Case 7
8	'97-S. 040	Human (M, 9)	Aug. 1997	Not tested	SM	Case 7
9	'97-S. 041	Human (F, 6)	Aug. 1997	Not tested	SM	Case 9
10	'97-S. 047	Human (F, 3)	Sept. 1997	Not tested	None	Case 3
11	'97-S. 048	Human (M, 8)	Sept. 1997	Not tested	SM	Case 1
12	'97-S. 051	Human (M, 8)	Sept. 1997	Not tested	TC	Case 3
13	'97-S. 052	Human (M, 4)	Oct. 1997	Not tested	SM	Case 3
14	'97-S. 055	Human (F, 6)	Oct. 1997	Not tested	SM	Case 14
15	'97-S. 064	Human (F, 4)	Nov. 1997	Not tested	None	Case 1
16	'97-S. 069	Human (M, 5)	Nov. 1997	Not tested	None	Case 3
17	'97-S. 071	Human (M, 11)	Nov. 1997	Not tested	None	Case 3
18	'98-S. 015	Human (F, 2)	May 1998	Not tested	None	Case 3
19	'98-S. 018	Human (M, 3)	May 1998	Not tested	None	Case 3
20	'98-S. 023	Human (M, 1)	June 1998	Not tested	ABPC	Case 3
21	'98-S. 024	Human (M, 7)	June 1998	Not tested	None	Case 3
22	'98-S. 029	Human (M, 3)	June 1998	Not tested	SM	Case 22
23	'98-S. 031	Human (M, 3)	July 1998	Not tested	SM	Case 22
24	'98-S. 035	Human (F, 9)	June 1998	Not tested	ABPC	Case 3
25	'98-S. 036	Human (F, 5)	Aug. 1998	Not tested	ABPC	Case 25 $\approx$ Case 3 <sup>3)</sup>
26	'98-S. 045	Human (F, 5)	Sept. 1998	Not tested	SM	Case 26
27	'98-S. 048	Human (M, 14)	Sept. 1998	Not tested	None	Case 3
28	'98-S. 058	Human (F, 2)	Oct. 1998	Not tested	None	Case 3
29	'98-S. 059	Human (M, 5)	Oct. 1998	Not tested	None	Case 3
30	'98-S. 063	Human (M, 2)	Nov. 1998	Not tested	None	Case 3
31	'98-S. 071	Human (F, 8)	Dec. 1998	Not tested	None	Case 3
32	Hyogo-SE100	Human (M, 11)	May 1999	4	None	Case 3
33	Hyogo-SE101	Human (F, 3)	June 1999	1	SM	Case 33
34	Hyogo-SE102	Human (M, 1)	July 1999	6	None	Case 3
35	Hyogo-SE113	Human (M, 3)	Aug. 1999	6	None	Case 3
36	Hyogo-SE114	Human (M, 3)	Sept. 1999	1	SM	Case 36
37	Hyogo-SE115	Human (F, 2)	Sept. 1999	4	None	Case 3
38	Hyogo-SE155	Human (M, 4)	Nov. 1999	UT	None	Case 3
39	Hyogo-SE156	Human (M, 1)	Nov. 1999	6	None	Case 3
40	Hyogo-SE157	Human (F, 2)	Dec. 1999	RDNC	None	Case 3
41	Hyogo-SE158	Human (F, 6)	Jan. 2000	6a	ABPC, TC, SM	Case 41 $\approx$ Case 3 <sup>4)</sup>
42	Hyogo-SE159	Human (F, 7)	Feb. 2000	9b	None	Case 42
43	Hyogo-SE173	Human (M, 1)	July 2000	6	None	Case 3
44	Hyogo-SE175	Human (F, 2)	Aug. 2000	4	None	Case 3
45	Hyogo-SE176	Human (M, 2)	Aug. 2000	4	None	Case 3
46	Hyogo-SE183	Human (M, 1)	Sept. 2000	UT	SM	Case 42
47	Hyogo-SE184	Human (F, 5)	Oct. 2000	6	None	Case 3
48	Hyogo-SE185	Human (F, 11)	Sept. 2000	6	None	Case 48
49	Kakogawa-A02	Chicken	June 1997	1	ABPC, TC, SM, GM	Case 49 $\approx$ Case 3 <sup>5)</sup>
50	Kakogawa-A12	Chicken	June 1997	UT	TC, SM	Case 50
51	Hyogo-SE064	Chicken egg	Oct. 1998	1	TC, SM	Case 51
52	Hyogo-SE174	Chicken egg	Aug. 2000	4	None	Case 3
53	Hyogo-SE186	Human (F, 54)	Nov. 2000	6	None	Case 3
54	Hyogo-SE189	Human (M, 22)	Oct. 2000	4	None	Case 3
55	Hyogo-SE192	Chicken egg	Nov. 2000	UT	None	Case 3

<sup>1)</sup> In three cases, the other serotypes of *Salmonella* were also isolated; that is, *S. Typhimurium* in case No. 1, *S. Paratyphi-B* in case 11, and *S. Thompson* in case 35.

<sup>2)</sup> SM: streptomycin, TC: tetracycline, ABPC: ampicillin, GM: gentamicin.

<sup>3)</sup> The largest band (>630kb) had only a slightly upper location as compared to case 3 (see lane 4 in Fig. 1B).

<sup>4)</sup> Two bands smaller than 48.5kb different from case 3 were existed (lane 6 in Fig. 1B).

<sup>5)</sup> An extra band existed near 48.5kb as compared to case 3 (lane 11 in Fig. 1B).

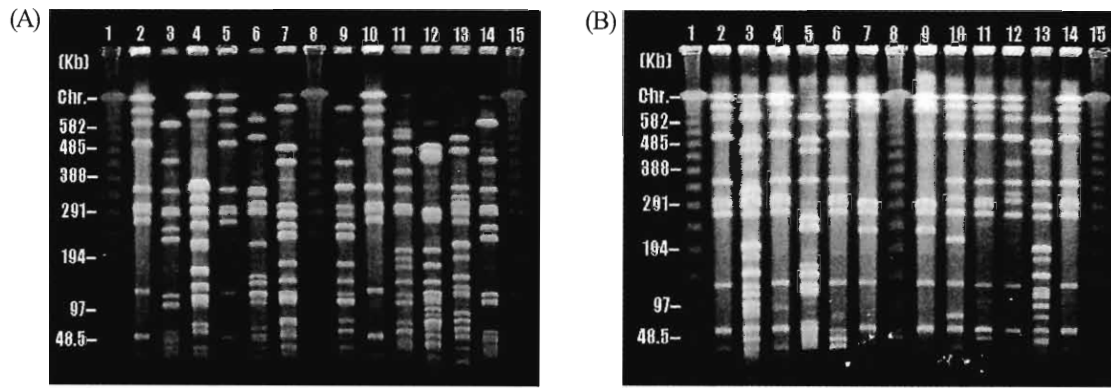


Fig.1. PFGE patterns of *BlnI*-digests of chromosomal DNA of *Salmonella* Enteritidis isolates from sporadic infections. (A) Lanes 1, 8, and 15:  $\lambda$  DNA ladder. Lane 2: Hyogo-SE186 (case 53). Lane 3: Tatsuno-03 (case 1). Lane 4: '96-S.070 (case 2). Lane 5: '96-S.076 (case 3). Lane 6: '97-S.001 (case 5). Lane 7: '97-S.039 (case 7). Lane 9: '97-S.041 (case 9). Lane 10: '98-S.048 (case 27). Lane 11: Hyogo-SE101 (case 33). Lane 12: Hyogo-SE114 (case 36). Lane 13: '97-S.055 (case 14). Lane 14: '97-S.064 (case 15). Chr.: chromosome. (B) Lanes 1, 8, and 15:  $\lambda$  DNA ladder. Lane 2: '97-S.069 (case 16). Lane 3: '98-S.029 (case 22). Lane 4: '98-S.036 (case 25). Lane 5: '98-S.045 (case 26). Lane 6: Hyogo-SE158 (case 41). Lane 7: Hyogo-SE159 (case 42). Lane 9: Hyogo-SE183 (case 46). Lane 10: Hyogo-SE185 (case 48). Lane 11: Kakogawa-A02 (case 49). Lane 12: Kakogawa-A12 (case 50). Lane 13: Hyogo-SE064 (case 51). Lane 14: Hyogo-SE189 (case 54). Chr: chromosome.

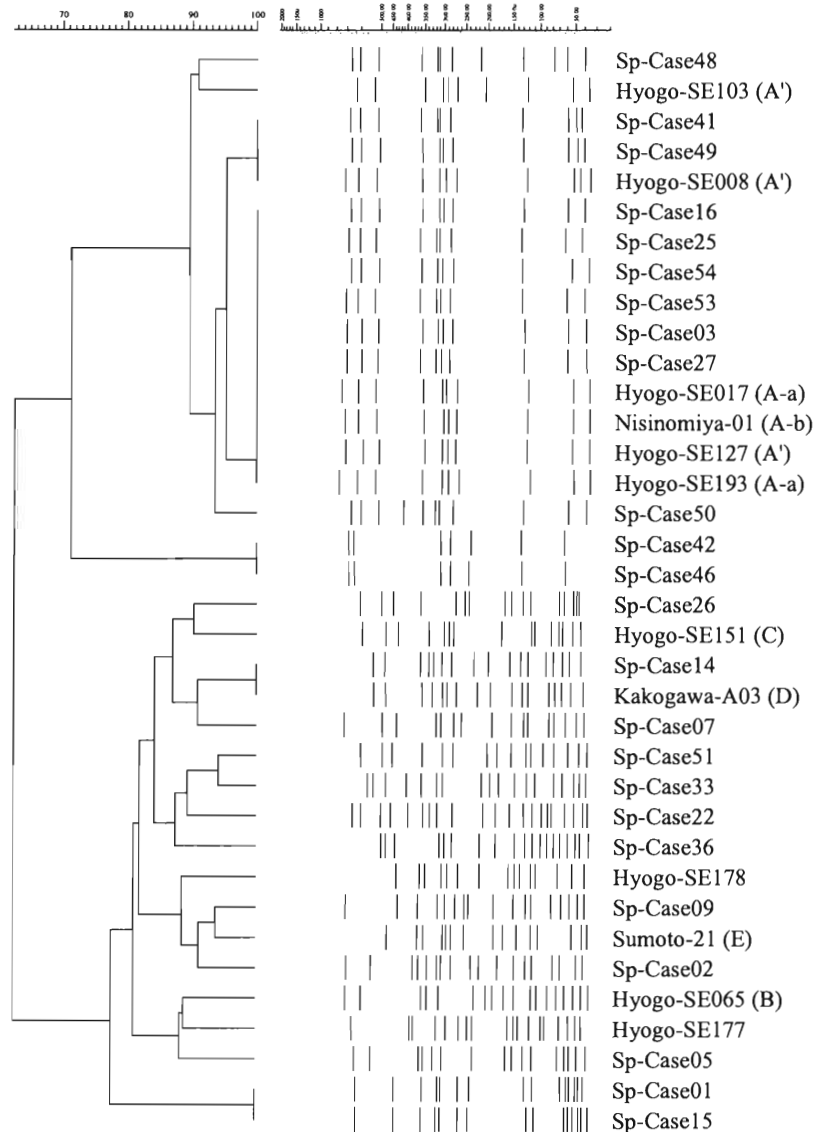


Fig.2. Cluster analysis of PFGE patterns of *Salmonella* Enteritidis shown in Figure 1 and the preceding one (6). For the analysis, the bands below 48.5 kb were omitted. Sporadic infections are indicated by SP-Case number. Cases from food poisonings were indicated by strain names showing genotypes in parentheses according to refs. 5, 6.

pattern was that seen in case 3 (at least 32 cases of the 55 cases) supposedly belonging to genotype A-b (6). There was no correlation between phage types and PFGE patterns (Table). The absence of correlation between PFGE patterns and phage types was previously reported for food poisoning cases (5,6). In summary, out of 55 isolates, 32 cases (about 60%) belonged to A-b (6), and 2 isolates (cases 41 and 49) had a pattern close to A-b. There were one isolate of A-a type (6) (case 25), one isolate similar to D type (5) (case 14), and 19 belonging to other nondescript genotypes (5,6).

Thus, the *Salmonella* Enteritidis isolates from various sporadic infections showed a great genetic diversity as in the cases of food poisoning outbreaks (5,6). This was clearly demonstrated by cluster analysis (Finger Printing Plus; Bio-Rad, Hercules, Calif., USA) (Fig. 2). The dendrograms indicated the presence of two large clusters, each containing several subclusters as reported for 28 food poisoning cases (6). The sporadic infections of *Salmonella* Enteritidis in Hyogo Prefecture over the past 4 years appear to have been caused by genotypically different bacteria as in food poisoning cases (6). The data appears to reflect the large-scale epidemic due to *Salmonella* Enteritidis since 1989 in Japan (1,2) including Hyogo Prefecture (3-6).

The authors are grateful to Drs. Hidemasa Izumiya and Haruo Watanabe, National Institute of Infectious Diseases, Tokyo, for the phage typing of our isolates and for a critical reading of the manuscript.

#### REFERENCES

1. National Institute of Health and Infectious Diseases Control, Ministry of Health and Welfare (1995): *Salmonella*, Japan, 1992-1994. Infect. Agents Surveillance Rep. 16, 1'-2'.
2. Terajima, J., Nakamura, A. and Watanabe, H. (1998): Epidemiological analysis of *Salmonella enterica* Enteritidis isolates in Japan by phage-typing and pulsed-field gel electrophoresis. Epidemiol. Infect., 120, 223-229.
3. Hamada, K., Tsuji, H., Shimada, K. and Hosoda, Y. (1999): Epidemiological analysis of *Salmonella* serovar Enteritidis isolates from two food poisoning outbreaks in Hyogo Prefecture by pulsed-field gel electrophoresis. Bull. Hyogo Prefect. Inst. Public Health, 34, 113-117 (in Japanese).
4. Tsuji, H., Shimada, K., Hamada, K. and Nakajima, H. (2000): Outbreak of *Salmonella* Enteritidis caused by contaminated buns peddled by a producer using traveling cars in Hyogo and neighboring Prefectures in 1999: an epidemiological study using pulsed-field gel electrophoresis. Jpn. J. Infect. Dis., 53, 23-24.
5. Hamada, K., Tsuji, H., Izumiya, H. and Watanabe, H. (2000): Comparison by pulsed-field gel electrophoresis of *Salmonella* Enteritidis genotypes from various food poisoning outbreaks from 1997 to 1999 in Hyogo Prefecture. Jpn. J. Infect. Dis., 53, 25-27.
6. Hamada, K. and Oshibe, T. (2001): 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. Jpn. J. Infect. Dis., 54, 38-40.
7. Hamada, K., Oshibe, T., Tsuji, H., Yoshida, S. and Aoki, Y. (2000): Outbreaks of heat stable enterotoxin-producing *Escherichia coli* O169 in the Kinki district in Japan: genotypic comparison by pulsed-field gel electrophoresis of isolates from two outbreaks in 2000 with isolates from four outbreaks in 1997-1998. Jpn. J. Infect. Dis., 53, 174-176.