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Phylogenetic Analysis of *Salmonella enterica* Serovar Enteritidis Isolated from Food Poisoning Outbreaks and Sporadic Infections in 2001-2002 in Hyogo Prefecture: Existence of Predominant Genotypes in the Epidemic

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Since 1989, *Salmonella enterica* serovar Enteritidis has become the most prevalent among the *Salmonella* isolates in Japan (1). We previously analyzed, by means of phylogenetic dendrograms of pulsed-field gel electrophoresis (PFGE), genetic variations of the *Salmonella* isolates from 28 food poisonings in 1997-2000 (2,3) and 55 cases of sporadic infections in 1996-2000 (4). In the present study, we analyzed isolates from 15 food poisonings and 12 sporadically infected cases in 2001-2002 and compared the results with those of the above cases in 1996-2000. We found predominant genotypes throughout the epidemics that took place over the past several

years in our prefecture.

We analyzed a total of 34 specimens; 22 isolates in 15 food poisoning outbreaks (15 from stool specimens and 7 from suspected foods) and 12 isolates (all from stool specimens) in 12 sporadic infections (Table 1). The identified phage types (PTs) of these isolates were PT1, PT1/1b, PT1c, PT4, PT6, PT14b, PT29, PT36, and PT47 (Table 1). As in previous cases (2-4), there was no correlation between PTs and PFGE patterns (see Table 1 and Figs. 1 and 2). Antibiotic sensitivity test using antibiotic disks (Becton Dickinson Microbiology Systems, Cockeysville, Md., USA) (5) revealed that all the

Table 1. Food poisoning outbreaks and sporadic infections of *Salmonella* Enteritidis (2001-2002) in Hyogo Prefecture and causative strains used in this paper

Case No.	Date of occurrence	Occasion	Exposed	Patients ¹⁾	Causative strains		
					Strain ²⁾	Phage type	Drug resistance ³⁾
29	May 2001	Meal at a grilled meat-restaurant	22	17*	Hyogo-SE197	4	None
30	June 2001	Funeral meal in a house	107	71	Hyogo-SE205	29	None
					Hyogo-SE212	4	None
31	June 2001	Meal in a home for the aged	89	15	Hyogo-SE236	4	None
32	June 2001	Meal in a home for the aged	100	25*	Hyogo-SE250	14b	None
					Hyogo-SE262**	14b	None
33	Aug. 2001	Lunch for delivery	60	44	Hyogo-SE263	1	SM
34	Aug. 2001	Meal at a restaurant at a university	263	56*	Hyogo-SE266	14b	None
35	Oct. 2001	Meal at an inn	114	60	Hyogo-SE285	14b	None
					Hyogo-SE292**	14b	None
36	Dec. 2001	Meal in a home for the aged	66	56	Hyogo-SE294	47	None
					Hyogo-SE314**	47	None
37	May 2002	Meal server by accommodations at a gymnastics meet	264	61	Hyogo-SE315	47	None
					Hyogo-SE325**	47	None
38	May 2002	Lunch at a nursery	66	35	Hyogo-SE327	1	SM
39	July 2002	Lunch at the restaurant in an office	18	17	Hyogo-SE349	36	None
40	July 2002	Meal at social welfare facilities	38	34	Hyogo-SE358	4	None
					Hyogo-SE378**	4	None
41	Aug. 2002	Meal at an inn	62	15	Hyogo-SE379	14b	None
42	Aug. 2002	Lunch at a nursery	89	18	Hyogo-SE389	6	None
					Hyogo-SE400**	6	None
43	Oct. 2002	Meal at a Chinese restaurant	10	7	Hyogo-SE405**	47	None

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Table 1.-Continued

Sp-56	Feb. 2001	Sporadic infection	Hyogo-SE196	47	None
Sp-57	Aug. 2001	Sporadic infection	Hyogo-SE281	1	SM
Sp-58	Aug. 2001	Sporadic infection	Hyogo-SE282	1	SM
Sp-59	Sept. 2001	Sporadic infection	Hyogo-SE283	47	None
Sp-60	Sept. 2001	Sporadic infection	Hyogo-SE284	1c	None
Sp-61	June 2002	Sporadic infection	Hyogo-SE347	1	None
Sp-62	June 2002	Sporadic infection	Hyogo-SE348	1	SM
Sp-63	June 2002	Sporadic infection	Hyogo-SE356	47	None
Sp-64	July 2002	Sporadic infection	Hyogo-SE357	1/1b	SM
Sp-65	Aug. 2002	Sporadic infection	Hyogo-SE402	1	SM
Sp-66	Aug. 2002	Sporadic infection	Hyogo-SE403	1	SM
Sp-67	Sept. 2002	Sporadic infection	Hyogo-SE404	4	None

¹⁾ * : One (cases 29 and 32) or three cooks (case 34) are involved in patients.

²⁾ ** : Isolates from causative foods.

³⁾ SM : Streptomycin.

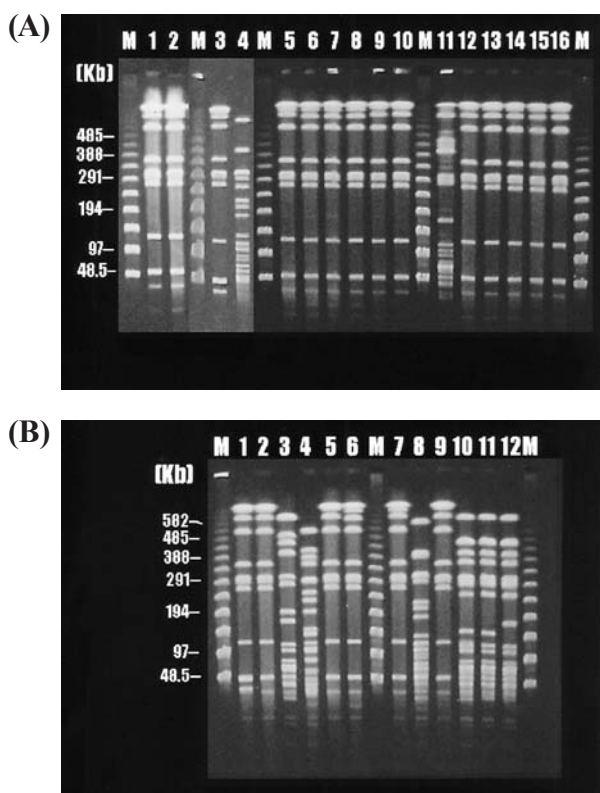


Fig. 1. PFGE patterns of *BlnI*-digests of chromosomal DNA of *Salmonella* Enteritidis isolates from food poisoning outbreaks (case-) (A) and sporadic infections (Sp-case-) (B). M: λ ladder.

(A) Lane 1: Hyogo-SE017 (case 6, genotype A-a). Lane 2: Nishinomiya-01(case 2, genotype A-b). Lane 3: Hyogo-SE315 (case 37). Lane 4: Hyogo-SE327 (case 38). Lane 5: Hyogo-SE197 (case 29). Lane 6: Hyogo-SE205 (case 30). Lane 7: Hyogo-SE212 (case 30). Lane 8: Hyogo-SE236 (case 31). Lane 9: Hyogo-SE250 (case 32). Lane 10: Hyogo-SE262 (case 32). Lane 11: Hyogo-SE263 (case 33). Lane 12: Hyogo-SE266 (case 34). Lane 13: Hyogo-SE285 (case 35). Lane 14: Hyogo-SE292 (case 35). Lane 15: Hyogo-SE294 (case 36). Lane 16: Hyogo-SE314 (case 36).

(B) Lane 1: Hyogo-SE325 (case 37). Lane 2: Hyogo-SE196 (Sp-case 56). Lane 3: Hyogo-SE281 (Sp-case 57). Lane 4: Hyogo-SE282 (Sp-case 58). Lane 5: Hyogo-SE283 (Sp-case 59). Lane 6: Hyogo-SE284 (Sp-case 60). Lane 7: Hyogo-SE347 (Sp-case 61). Lane 8: Hyogo-SE348 (Sp-case 62). Lane 9: Hyogo-SE356 (Sp-case 63). Lane 10: Hyogo-SE357 (Sp-case 64). Lane 11: Hyogo-SE402 (Sp-case 65). Lane 12: Hyogo-SE403 (Sp-case 66).

isolates were sensitive to ampicillin, cefotaxime, kanamycin, gentamicin, streptomycin (SM), tetracycline, trimethoprim, ciprofloxacin, fosfomycin, chloramphenicol, sulphamethoxazole-trimethoprim, and nalidixic acid except eight isolates sensitive to all but SM (Table 1).

We analyzed the PFGE patterns of the isolates employing a Gene Path Typing System (Program No. 2; Bio-Rad Laboratories, Hercules, Calif., USA). As shown in Fig. 1, the PFGE patterns of bacterial chromosomal DNAs digested with *BlnI* (Takara Shuzo, Co., Ltd., Kyoto) resembled the A types, A-a and A-b, observed in the past (3). A-a and A-b were quite similar; their only difference was that a >630 kb band in A-a (see Hyogo-SE017, lane 1 in Fig. 1A) migrated slightly more slowly than that in A-b (see Nishinomiya-01, lane 2 in Fig. 1A). In the 15 food poisoning cases (Fig. 1A), 13 were caused by A types. Only two, Hyogo-SE327 from case 38 (lane 4) and Hyogo-SE263 from case 33 (lane 11) were different. In contrast, among 12 sporadic cases (Fig. 1B), six cases, Sp-cases 57, 58, 62, 64, 65, and 66 (lanes 3, 4, 8, 10, 11, and 12, respectively) were not A types.

A cluster analysis (Finger Printing Plus; Bio-Rad) of the present isolates and those in the past years, 1996-2000 (2-4), together (Fig. 2) indicated presence of two large clusters with low similarity (approximately 60%): a main cluster (cluster I) including A-a, A-b, and three A' (2) types (see the footnote in Fig. 2) and a minor one (cluster II) constituted of unique genotypes such as B-E types (2) (see the footnote in Fig. 2). The cluster I was homogeneous except for two isolates, Sp-case 42 and Sp-case 46, that probably constitute a sub-cluster. Cluster II consisted of several sub-clusters.

Our data clearly showed that most food poisoning outbreaks and half of the sporadic cases in 1996-2002 were caused by *S. enterica* serovar Enteritidis whose genotype belongs to cluster I. This observation is largely different from that regarding the enterohemorrhagic *Escherichia coli* O157 epidemic in Japan since 1996, which rarely showed predominant genotypes throughout the epidemic (6). There was no correlation between PTs and PFGE patterns in the *S. Enteritidis* epidemic as indicated here and previously (2-4).

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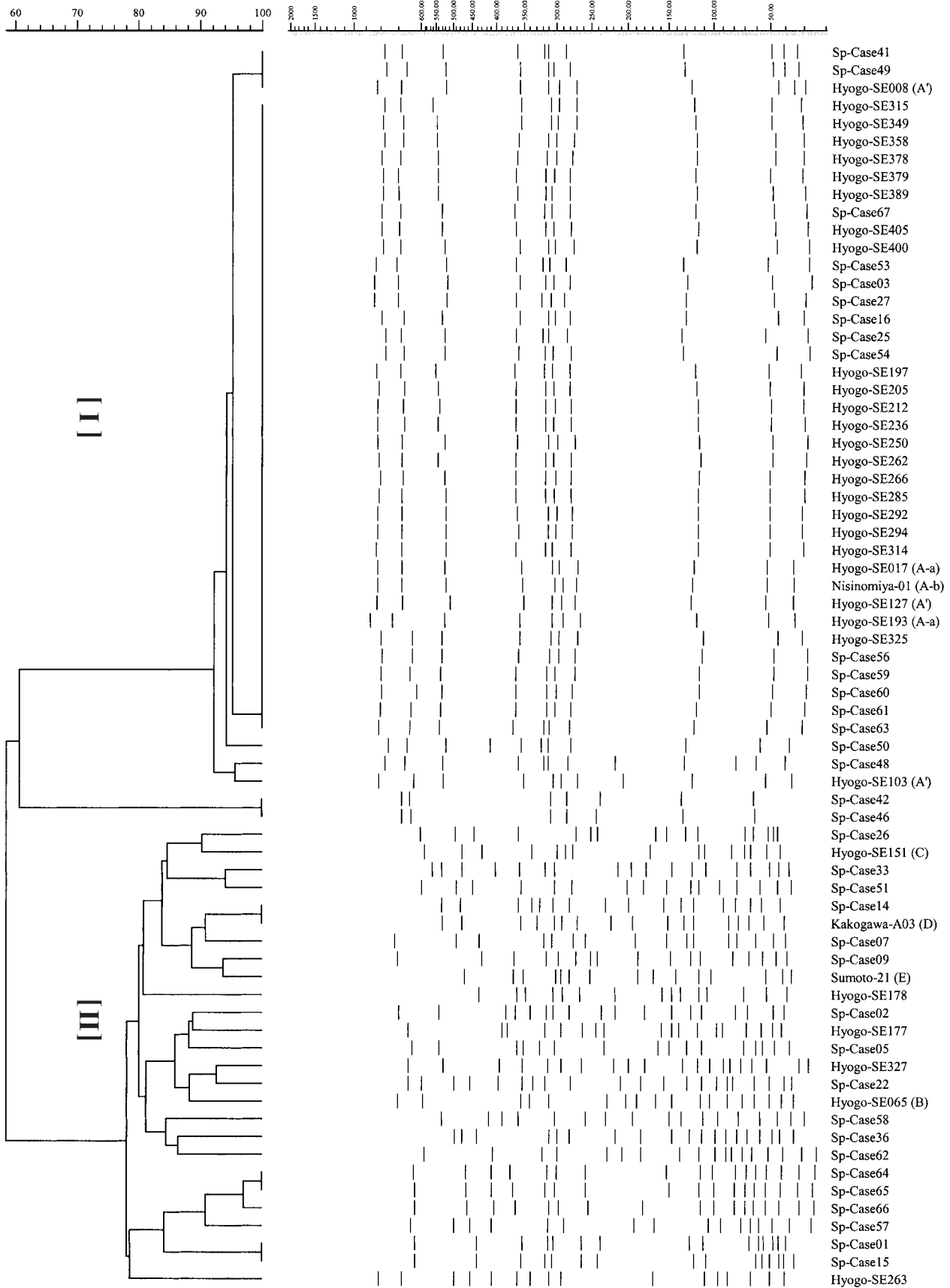


Fig. 2. Cluster analysis of PFGE patterns of *Salmonella* Enteritidis isolates reported in this and previous reports (2-4). Cases from food poisonings are indicated by strain names. Sporadic infections are indicated by Sp-case numbers. Genotypes in parentheses in some cases are shown according to refs. 2 and 3. The genotype A and subtypes A-a and A-b are described in the text. The PFGE patterns similar to A but having an extra band unique to each strain are coded as A'. There were three such patterns with an extra band of 194-243 kb (Hyogo-SE103, case 14), with an extra band just below 48.5 kb (Hyogo-SE008, case 5), and with an extra band far below 48.5 kb (Hyogo-SE127, case 17). For analysis, the bands below 48.5 kb were not taken significantly into account. The B-E genotypes showed unique PFGE patterns.

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