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Comparison by Pulsed-Field Gel Electrophoresis of *Salmonella* Enteritidis Genotypes from Various Food Poisoning Outbreaks from1997 to 1999 in Hyogo Prefecture

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Salmonella serovar Enteritidis has become the most prevalent among the *Salmonellae* serotypes in Japan since 1989 (1). From 1997 to 1999, Hyogo Prefecture experienced 21 known food poisoning outbreaks caused by the serovar. To determine the genetic characters of the strains involved in Hyogo Prefecture, we determined the phage types and genotypes of representative isolates from the 21 outbreaks (2, 3) (Table).

The 21 isolated strains showed various phage types (PTs), such as 1, 4, 5, 6, 6a, 34, and RDNC (Reaction Does Not Conform). All were prevalent in Japan (1), but RDNC (4) emerged abruptly in 1998 in Hyogo Prefecture. They were examined by pulsed-field gel electrophoresis (PFGE) by using a Gene Path Typing System (Nippon Bio-Rad, Tokyo) (Fig. 1, 2). There were five PFGE patterns revealed by the *Bln*I chromosomal DNA digests. There was no correlation between PFGE pattern and phage type.

A major PFGE pattern, group A, exhibited one PT1 (Hyogo-

Table. Food poisoning outbreaks of *Salmonella* Enteritidis (1997-1999) in Hyogo Prefecture and causative strains used in this paper

Case No.	Date	Occasion	Exposed	Patients	Causative strain used			Reference
					Strain	Phage type	PFGE pattern by *Bln*I	
1	June 1997	Lunch at a primary school	664	129	Kakogawa-A03	34	D	(2)
2	Dec. 1997	Dinner at home (egg)	2	2	Nishinomiya-01	4	A	
3	Feb. 1998	Funeral meal at an inn	80	32	Sumoto-21	4	E	
4	Feb. 1998	Lunch and/or employees at a nursery	~96	6	Hyogo-SE005	1	A	(2)
5	July 1998	Lunch at a hotel	41	7	Hyogo-SE008	6	A'	
6	Aug. 1998	Rolled sushi from a supermarket	Unknown	9	Hyogo-SE017	RDNC	A	
7	Aug. 1998	Egg from a store at home	Unknown	1	Hyogo-SE026	RDNC	A	
8	Oct. 1998	Lunch at a nursery	75	30	Hyogo-SE028	RDNC	A	
9	Nov. 1998	Funeral meal at home (egg dish)	111	42	Hyogo-SE058	4	A	
10	Nov. 1998	Dinner at a hotel	15	11	Hyogo-SE065	RDNC	B	
11	Dec. 1998	Lunch for delivery	48	5	Hyogo-SE072*	4	A	
12	Jan. 1999	Hospital supper	247	57	Hyogo-SE073	4	A	
13	Mar. 1999	In-hospital infection	Unknown	5	Hyogo-SE096	RDNC	A	
14	July 1999	Korean pancake from a market in 3 families	12	6	Hyogo-SE103	6a	A'	
15	Aug. 1999	Chinese noodles at a restaurant	2	2	Hyogo-SE106	RDNC	A	
16	Sept. 1999	Cream bun sold by traveling cars in Hyogo and 2 Prefectures	Unknown	206	Hyogo-SE116	RDNC	A	(3)
17	Oct. 1999	Omelet with rice from a super-market at home	4	4	Hyogo-SE127	5	A'	
18	Aug. 1999	Dinner at home	5	5	Hyogo-SE138	4	A	
19	Sept. 1999	Sushi for delivery from the same sushi-bar in 3 school field days and a wheel-chair marathon race	246	41	Hyogo-SE129	RDNC	A	
20	Sept. 1999	Dinner with a family at an Indian restaurant and/or employees there	7	7	Hyogo-SE151	1	C	
21	Sept. 1999	Hospital supper	29	8	Hyogo-SE143	RDNC	A	

*A separete experiment indicated that the PFGE patterns with both *Bln*I and *Xba*I were essentially the same as those of Hyogo-SE058 in case 9.

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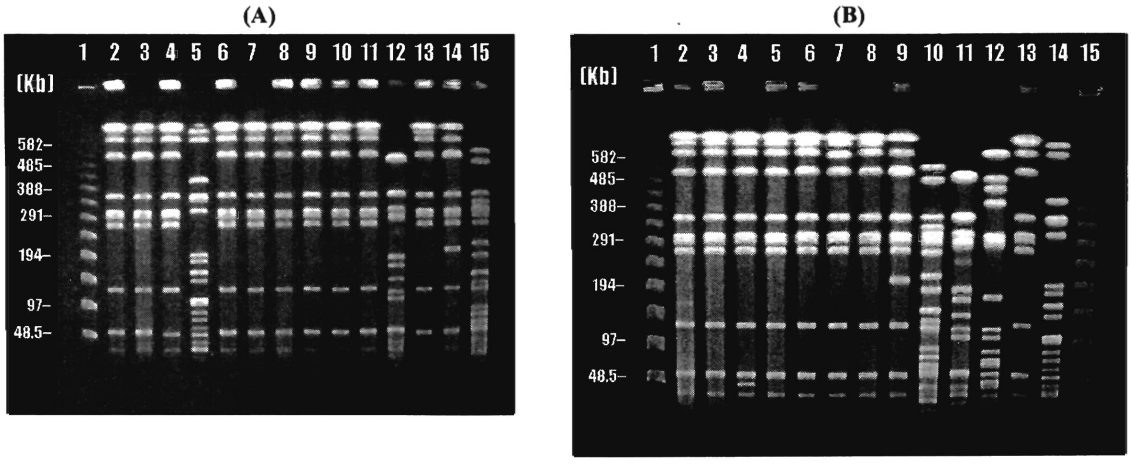

Fig. 1. PFGE pattern of *Bln*I-digests of chromosomal DNA of *Salmonella* Enteritidis isolates.
(A) *Salmonella* Enteritidis isolates with RDNC phage types.
Lane 1; λ DNA ladder. Lane 2; Hyogo-SE017 (RDNC), case 6. Lane 3; Hyogo-SE026 (RDNC), case 7. Lane 4; Hyogo-SE028 (RDNC), case 8. Lane 5; Hyogo-SE065 (RDNC), case 10. Lane 6; Hyogo-SE096 (RDNC), case 13. Lane 7; Hyogo-SE106 (RDNC), case15. Lane 8; Hyogo -SE116 (RDNC), case16. Lane 9; Hyogo-SE129 (RDNC), case 19. Lane 10; Hyogo-SE143 (RDNC), case 21. Lane 11; Hyogo-SE005 (PT1), case 4. Lane 12; Sumoto-21 (PT4), case 3. Lane 13; Hyogo-SE073 (PT4), case 12. Lane 14; Hyogo-SE103 (PT6a), case 14. Lane 15; Kakogawa-A03 (PT34), case 1.
(B) *Salmonella* Enteritidis isolates with other phage types.
Lane 1, 15; λ DNA ladder. Lane 2; Nishinomiya-01 (PT4), case 2. Lane 3; Hyogo-SE005 (PT1), case 4. Lane 4; Hyogo-SE008 (PT6), case 5. Lane 5; Hyogo-SE058 (PT4), case 9. Lane 6; Hyogo-SE073 (PT4), case 12. Lane 7; Hyogo-SE127 (PT5), case17. Lane 8; Hyogo-SE138 (PT4), case18. Lane 9; Hyogo-SE103 (PT6a), case 14. Lane 10; Kakogawa-A03 (PT34), case 1. Lane 11; Sumoto-21 (PT4), case 3. Lane 12; Hyogo-SE151 (PT1), case 20. Lane 13; Hyogo-SE017 (RDNC), case 6. Lane 14; Hyogo-SE065 (RDNC), case 10.

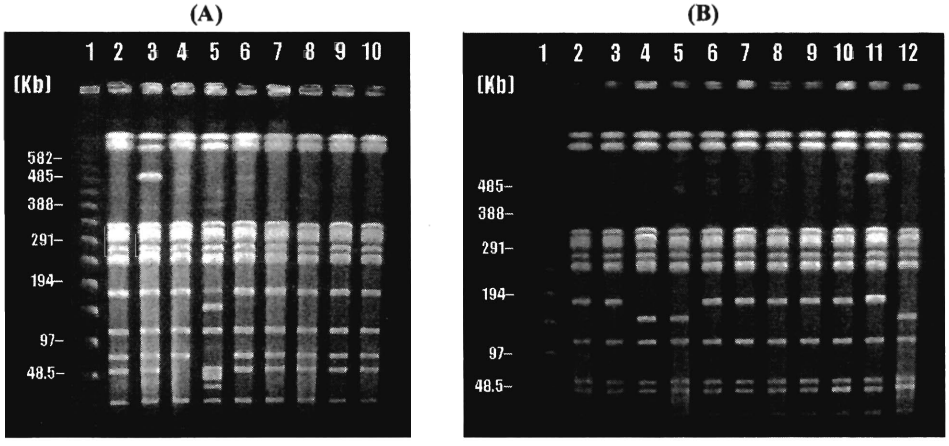

Fig. 2. PFGE pattern of *Xba*I-digests of chromosomal DNA of *Salmonella* Enteritidis isolates.
(A) *Salmonella* Enteritidis isolates with RDNC phage types.
Lane 1; λ DNA ladder. Lane 2; Hyogo-SE017, case 6. Lane 3; Hyogo-SE026, case 7. Lane 4; Hyogo-SE028, case 8. Lane 5; Hyogo-SE065, case 10. Lane 6; Hyogo-SE096, case 13. Lane 7; Hyogo-SE106, case15. Lane 8; Hyogo-SE116, case16. Lane 9; Hyogo-SE129, case 19. Lane 10; Hyogo-SE143, case 21.
(B) *Salmonella* Enteritidis isolates with other phage types.
Lane 1; λ DNA ladder. Lane 2; Nishinomiya-01 (PT4), case 2. Lane 3; Hyogo-SE005 (PT1), case 4. Lane 4; Hyogo-SE151 (PT1), case 20. Lane 5; Sumoto-21 (PT4), case 3. Lane 6; Hyogo-SE058 (PT4), case 9. Lane 7; Hyogo-SE073 (PT4), case12. Lane 8; Hyogo -SE138 (PT4), case 18. Lane 9; Hyogo-SE127 (PT5), case 17. Lane 10; Hyogo-SE008 (PT6), case 5. Lane 11; Hyogo-SE103 (PT6a), case 14. Lane 12; Kakogawa-A03 (PT34), case 1.

SE005, lane 11 in Fig. 1A and lane 3 in Fig. 1B), five PT4s (Nishinomiya-01, lane 2 in Fig. 1B; Hyogo-SE058, lane 5 in Fig. 1B; Hyogo-SE073, lane 13 in Fig. 1A and lane 6 in Fig. 1B; Hyogo-SE138, lane 8 in Fig. 1B; and Hyogo-072, data not shown), and eight RDNCs (Hyogo-SE017, lane 2 in Fig. 1A and lane 13 in Fig. 1B; Hyogo-SE026, lane 3 in Fig. 1A; Hyogo-SE028, lane 4 in Fig. 1A; Hyogo-SE096, lane 6 in Fig. 1A; Hyogo-SE106, lane 7 in Fig. 1A; Hyogo-SE116, lane 8 in Fig. 1A; Hyogo-SE129, lane 9 in Fig. 1A; and Hyogo-SE143, lane 10 in Fig. 1A). The PFGE pattern quite similar to group A but having an extra band unique to each strain was named A'. It consisted of one PT6 (Hyogo-SE008, case 5, lane 4 in Fig. 1B) with an extra band just below 48.5 kb, one PT6a (Hyogo-SE103, case 14, lane 14 in Fig. 1A and lane 9 in Fig. 1B) with an extra band of 194 - 243 kb, and one PT5 (Hyogo-SE127, case 17, lane 7 in Fig. 1B) with an extra band far below 48.5 kb. In groups A and A', all the non-RDNC strains except Hyogo-SE103 (case 14) had a common extra band slightly larger than 630 kb.

The remaining four strains exhibited unique PFGE patterns.

They were classified as groups B-E; a strain of RDNC, Hyogo-SE065 (case 10) as group B (Fig. 1A, lane 5 and Fig. 1B, lane 14); a strain of PT1, Hyogo-SE151 (case 20) as group C (Fig. 1B, lane 12); a strain of PT34, Kakogawa-A03 (case 1) as group D (Fig. 1B, lane 10); and a strain of PT4, Sumoto-21 (case 3) as group E (Fig. 1A, lane 12 and Fig. 1B, lane 11). The PFGE patterns of *Xba*I digests were similar for all the isolates (Fig. 2). The PFGE pattern grouped as above for each isolate is shown in Table together with other descriptions.

We have thus described a PFGE analysis of *Salmonella* Enteritidis from the recent 21 food poisoning outbreaks. Five distinct patterns were detected upon PFGE. No correlation with phage type was detected.

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