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Phylogenetic Analysis of Genotypic Variations of *Escherichia coli* O157:H7 Isolates from Sporadic Infections by Using Pulsed-Field Gel Electrophoresis from March 1999 to February 2001 in Hyogo Prefecture

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Entero-hemorragic *Escherichia coli* (EHEC) is a highly virulent enteric pathogen and, since 1982, it has been found worldwide including the United States, Europe, and Japan (1). *E. coli* O157:H7 has been the most prevalent serotype among them (1). A large-scale outbreak occurred in 1996 in Sakai City in Osaka Prefecture (2). The EHEC epidemic has continued ever since (3). Even in the early epidemics, its high genetic diversity was revealed by pulsed-field gel electrophoresis (PFGE) (2). We previously reported such genotypic variations in 1997-1998 isolates (4). We report here the genetic diversity and phylogenetic analysis of the isolates in 1999-early 2001. Eighty isolates from human feces of sporadic and small outbreak cases (Table) and one isolate from well water implicated in a familial transmission were analyzed.

The isolates were examined for genes encoding verotoxin (VT) by polymerase chain reaction (PCR) using EVS- (for VT1) and EVT- (for VT2) primers purchased from Takara Shuzo Co., Ltd., (Kyoto) (5). Six isolates were positive for

Table. Infectional cases in small groups by *Escherichia coli* O157:H7 from 1999 to early 2001 in Hyogo Prefecture and some characters of the isolates[1]

Case No.	Sampling date	Relationships among patients	Patient No.	Strain[2]	VT[3] type produced	Drug resistance[4]
1	March 1999	family members	A1 (male, 4 y)	'98-E.086	VT 1, 2	SM, TC
			A2 (female, 67 y)	'98-E.087	VT 1, 2	SM, TC
2	June 1999	family members	A3 (female, 5 y)	'99-E.004	VT 2	None
		except A6, a friend of A3 and	A4 (male, 1 y)	'99-E.005	VT 2	None
		A7, well water[5]	A5 (male, 35 y)	'99-E.006	VT 2	None
			A6 (female, 6 y)	'99-E.007	VT 2	None
			A7	'99-E.008	VT 2	None
			A8 (male, 8 y)	'99-E.009	VT 2	None
3	July 1999	day nursery children except A14.	A9 (male, 3 y)	'99-E.011	VT 1	SM, TC
		A12, A13 and A14 were from the	A10 (male, 2 y)	'99-E.012	VT 1	SM, TC
		same family.	A11 (male, 2 y)	'99-E.013	VT 1	SM, TC
			A12 (male, 2 y)	'99-E.014	VT 1	SM, TC
			A13 (female, 4 y)	'99-E.015	VT 1	SM, TC
			A14 (female, 7 y)	'99-E.016	VT 1	SM, TC
4	August 1999	family members	A15 (female, 57 y)	'99-E.024	VT 1, 2	ABPC, SM, TC
			A16 (male, 4 y)	'99-E.025	VT 1, 2	ABPC, SM, TC
5	September 1999	employees in a high school canteen	A17 (female, 60 y)	'99-E.030	VT 2	None
			A18 (female, 65 y)	'99-E.031	VT 2	None
6	October 1999	employees in a kindergarten canteen	A19 (female, 32 y)	'99-E.033	VT 2	None
			A20 (female, 52 y)	'99-E.034	VT 2	None
7	April 2000	family members	B1 (female, 62 y)	'00-E.001	VT 2	None
			B2 (male, 31 y)	'00-E.002	VT 2	None
8	July 2000	family members	B3 (male, 6 y)	'00-E.049	VT 2	ABPC
			B4 (male, 4 y)	'00-E.050	VT 2	ABPC
9	August 2000	family members	B5 (female, 44 y)	'00-E.055	VT 2	None
			B6 (male, 1 y)	'00-E.056	VT 2	None
10	August 2000	family members	B7 (male, 2 y)	'00-E.057	VT 2	None
			B8 (female, 5 y)	'00-E.058	VT 2	None
11	October 2000	family members	B9 (female, 9 y)	'00-E.068	VT 2	None
			B10 (female, 4 y)	'00-E.069	VT 2	None

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Table-Continued

Case No.	Sampling date	Relationships among patients	Patient No.	Strain[2]	VT[3] type produced	Drug resistance[4]
12	October 2000	family members	B11 (male, 9 y)	'00-E.071	VT 1, 2	None
			B12 (female, 37 y)	'00-E.072	VT 1, 2	None
			B13 (male, 7 y)	'00-E.073	VT 1, 2	None
13	October 2000	family members	B14 (female, 50 y)	'00-E.075	VT 2	None
			B15 (female, 19 y)	'00-E.078	VT 2	None
14	November 2000	residents in a home for the aged (B16, B19, and B20) and family members of B16 (B17 and B18)	B16 (female, 88 y)	'00-E.081	VT 1, 2	None
			B17 (male, 63 y)	'00-E.082	VT 1, 2	None
			B18 (female, 58 y)	'00-E.083	VT 1, 2	None
			B19 (female, 95 y)	'00-E.084	VT 1, 2	None
			B20 (female, 86 y)	'00-E.085	VT 1, 2	None
15	February 2001	family members	C1 (female, 2 y)	'00-E.087	VT 1, 2	None
			C2 (male, 59 y)	'00-E.088	VT 1, 2	ABPC

[1] All the isolated bacteria were from human feces unless otherwise indicated. Bacteria were identified only in cases in which more than two strains were obtained.
[2] The isolates in each case except A4, A6, A16, A20, B6, and B19 showed the same pattern on PFGE (see Fig. 2). The PFGE analysis of two strains in case No. 1 was unsuccessful due to DNA degradation.
[3] Verotoxin.
[4] ABPC: ampicillin, SM: streptomycin, TC: tetracycline.
[5] Well water used for purposes other than drinking water.

VT1, 41 for VT2, and 34 for the both (Table). Sensitivities to 12 antibiotics, i.e., ABPC (ampicillin), CTX (cefotaxime), KM (kanamycin), GM (gentamicin), SM (streptomycin), TC (tetracycline), TMP (trimethoprim), CPFX (ciplofloxacin), FOM (fosfomycin), CP (chloramphenicol), ST (sulfamethoxazole-trimethoprim), and NA (nalidixic acid) were examined by using Sensi Disk (Nippon Becton Dickinson Co., Ltd., Tokyo) (6). Sixty-two isolates were sensitive to all the antibiotics examined, but the remaining 19 strains were resistant to either ABPC (6 strains), SM (1 strain), ABPC+SM (1 strain), SM+TC (9 strains), or ABPC+SM+TC (2 strains) (Table).

PFGE patterns of the 81 isolates were analyzed by using a gene path typing system (Program No. 5 or No. 23; Nippon Bio-Rad, Tokyo) as reported previously (7). The typical PFGE patterns are shown in Figs. 1. The PFGE patterns of *Xba*I-digested chromosomal DNA of the 81 isolates and cluster analysis (Finger Printing PLUS, Bio-Rad, Hercules, Calif., USA) of the 72 strains (Fig. 2) revealed a large strain-to-strain or case-to-case variation. The dendrogram indicated

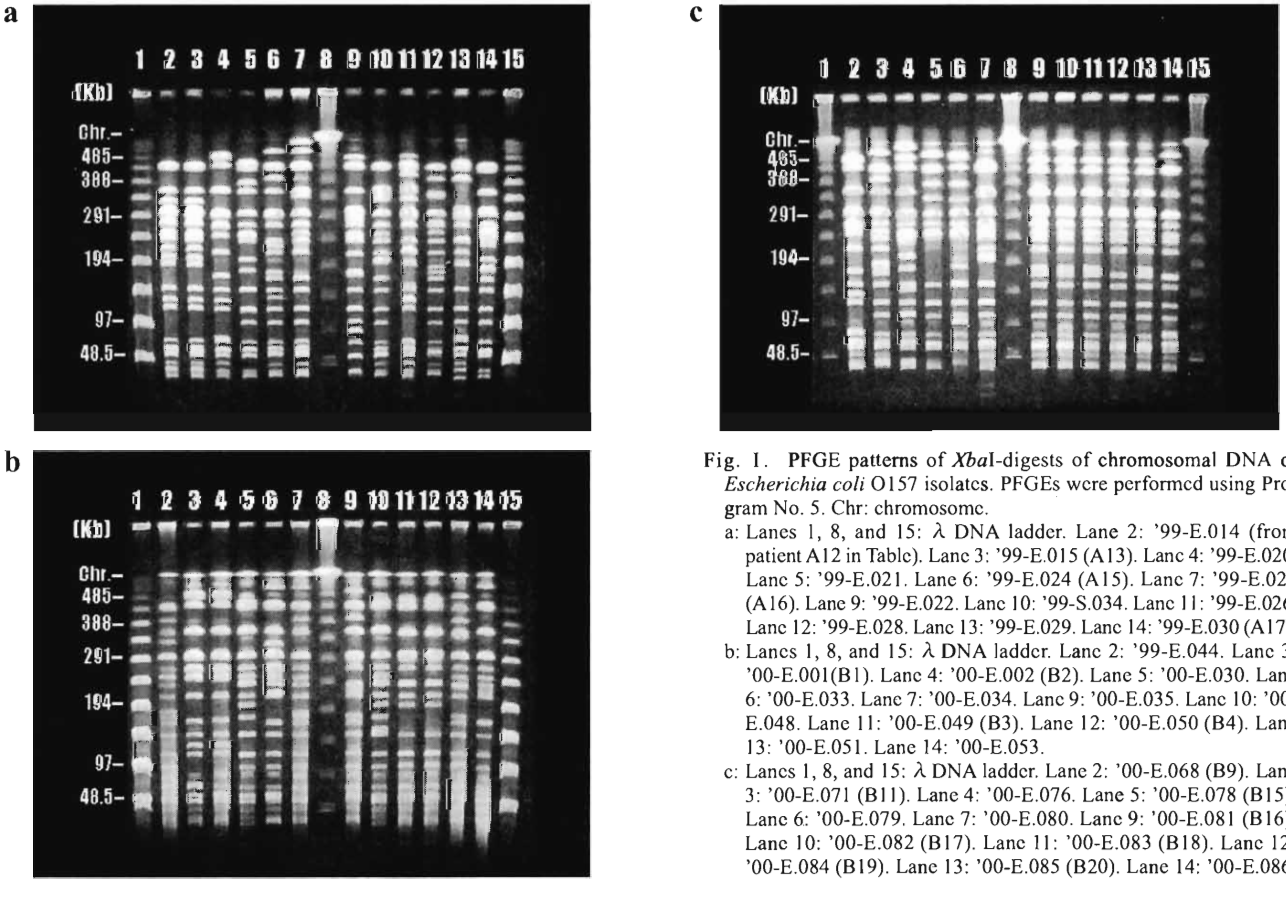

Fig. 1. PFGE patterns of *Xba*I-digests of chromosomal DNA of *Escherichia coli* O157 isolates. PFGEs were performed using Program No. 5. Chr: chromosome.
a: Lanes 1, 8, and 15: λ DNA ladder. Lane 2: '99-E.014 (from patient A12 in Table). Lane 3: '99-E.015 (A13). Lane 4: '99-E.020. Lane 5: '99-E.021. Lane 6: '99-E.024 (A15). Lane 7: '99-E.025 (A16). Lane 9: '99-E.022. Lane 10: '99-S.034. Lane 11: '99-E.026. Lane 12: '99-E.028. Lane 13: '99-E.029. Lane 14: '99-E.030 (A17).
b: Lanes 1, 8, and 15: λ DNA ladder. Lane 2: '99-E.044. Lane 3: '00-E.001(B1). Lane 4: '00-E.002 (B2). Lane 5: '00-E.030. Lane 6: '00-E.033. Lane 7: '00-E.034. Lane 9: '00-E.035. Lane 10: '00-E.048. Lane 11: '00-E.049 (B3). Lane 12: '00-E.050 (B4). Lane 13: '00-E.051. Lane 14: '00-E.053.
c: Lanes 1, 8, and 15: λ DNA ladder. Lane 2: '00-E.068 (B9). Lane 3: '00-E.071 (B11). Lane 4: '00-E.076. Lane 5: '00-E.078 (B15). Lane 6: '00-E.079. Lane 7: '00-E.080. Lane 9: '00-E.081 (B16). Lane 10: '00-E.082 (B17). Lane 11: '00-E.083 (B18). Lane 12: '00-E.084 (B19). Lane 13: '00-E.085 (B20). Lane 14: '00-E.086.

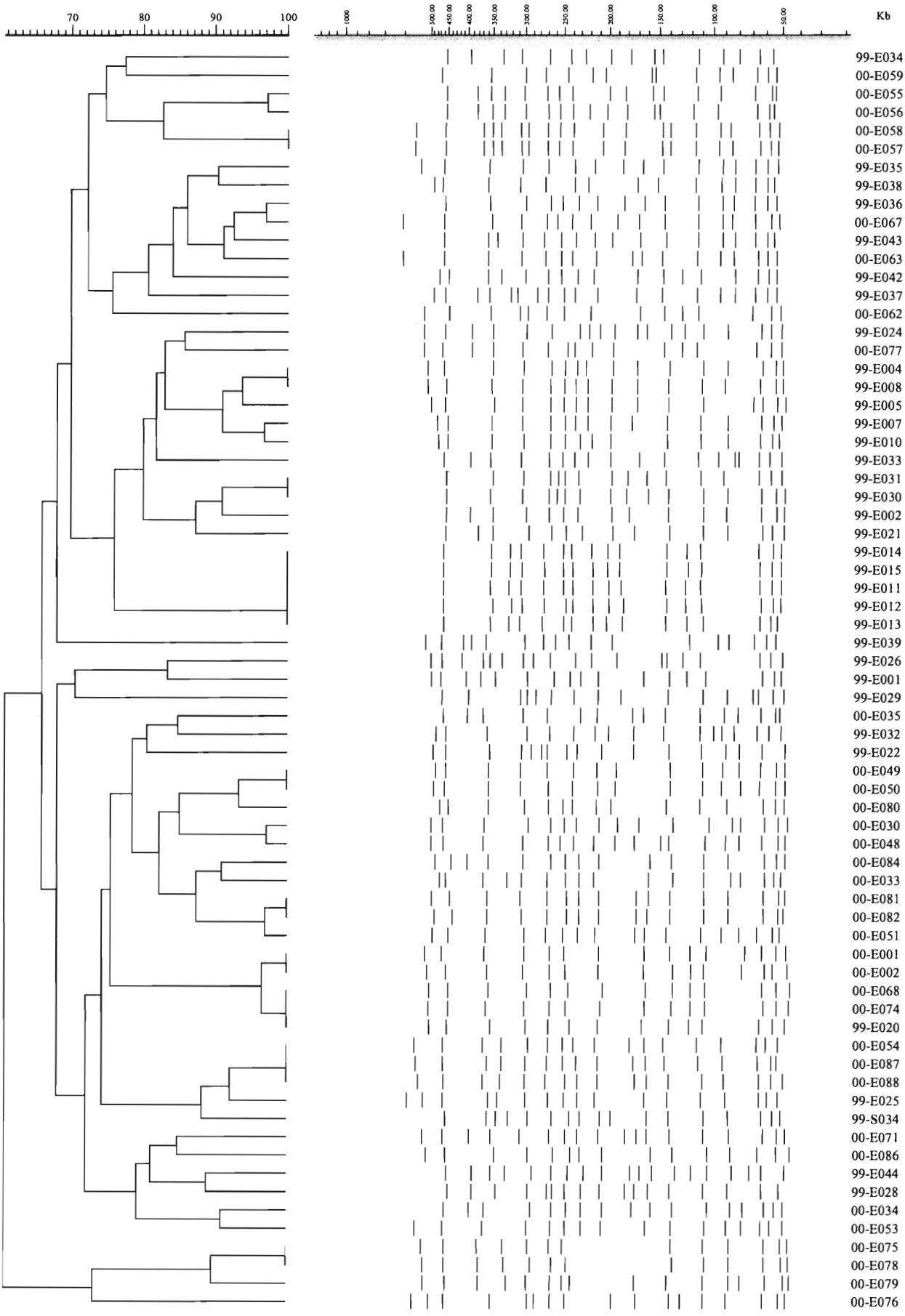

Fig. 2. Cluster analysis of PFGE patterns of *Escherichia coli* O157 isolates. From 72 strains to be analyzed on PFGE figures, three having degraded patterns were omitted. For the analysis, the bands far below 48.5 kb were omitted.

the presence of more than ten small clusters. Thus, it was concluded that EHEC O157:H7 epidemics in Hyogo Prefecture in the past 2 years were caused by various EHECs of different genotypes.

The question arises as to why *E. coli* O157 is so genetically changeable and what advantage this characteristic lends to bacteria. Emergence and rapid spread of this organism can be explained by a high incidence of highly mutable variants which are found among isolates of EHEC and *Salmonella enterica*; they may cause enhanced genetic variability of a population and accelerate adaptive evolution (8). But, this may not explain the observation that *E. coli* O157 showed a larger PFGE diversity than did *Salmonella* Enteritidis (9,10). However, it should be mentioned that variability of PFGE patterns is influenced by the number and mutability of the sites recognized by the restriction enzymes. Therefore, the above mentioned different variability could be more apparent than real.

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