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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

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An outbreak of enterotoxigenic *Escherichia coli* serotype O169:H41 infection was first reported in Japan in 1991 (1,2). From June 1997 to August 1998, we investigated four outbreaks (cases A-D) due to the same organism in Hyogo and neighboring Prefectures (3). In 2000, two more outbreaks occurred.

One outbreak occurred at the International Gardening and Landscaping Exhibition Japan Flora 2000 at Awaji Island in Hyogo Prefecture in May (case E). Two hundred and sixty-six persons among three package-tour groups from neighboring Prefectures ate a Japanese-style box lunch prepared by a hotel near the site of the exposition. About half of the members (121 persons) experienced watery diarrhea and/or abdominal pain. We identified *E. coli* O169:H41 in fecal specimens from 22 patients and from one of 19 cooks at the hotel. The other outbreak occurred in April 2000 in a reformatory in Nara Prefecture (case F). Seventy-three of 88 boys aged 16-20 years became ill with symptoms including diarrhea, abdominal pain,

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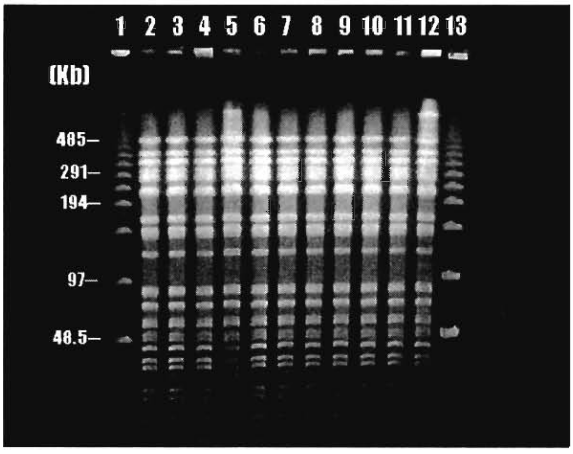

Fig.1. PFGE patterns of *Xba*I digests of chromosomal DNAs of *E. coli* O169:H41 isolated from case E outbreak (lanes 2-12). The PFGE was performed with non-linear program No.23. Lanes 1 and 13 are λ DNA ladders.

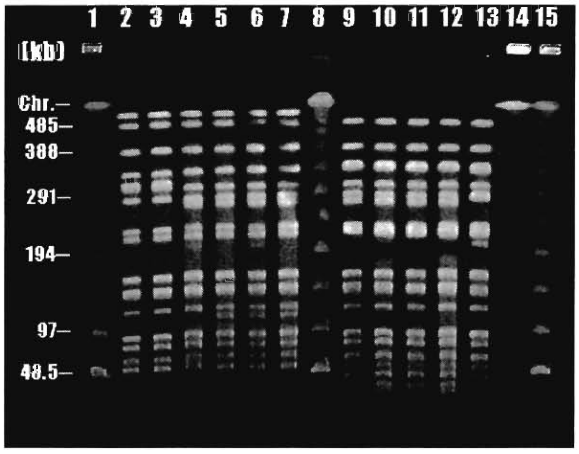

Fig.2. PFGE patterns of *Xba*I digests of chromosomal DNAs of *E. coli* O169:H41 isolates from various outbreaks. The PFGE was performed with linear program No.5. Lanes 1, 8, and 15: λ DNA ladders; lanes 2-3: isolates from patients in case A; lane 4: an isolate from patients in case D; lane 5: an isolate from patients in case B; lane 6: an isolate from the causative food, *wakame*, in case B; lane 7: an isolate from patients in case C; lanes 9-10: isolates from patients in case E; lanes 11-12: isolates from patients in case F; lane 13: an isolate from the traveler to India in 1999 as a reference unrelated to cases A-F; lane 14: the same bacteria as that in lane 7 with no treatment by *Xba*I. Chr: chromosome.

and vomiting. They had eaten lunch served by the institution. Twelve non-motile *E. coli* O169 isolates were obtained from fecal specimens.

The strains were examined for genes encoding LT, STh, and STp by polymerase chain reaction using primers purchased from Takara Shuzo Co. Ltd. (Kyoto). All of the isolates were negative for LT and STh, and positive for STp except for two strains. The two STp negatives probably had lost the toxin-encoding Ent plasmid (4) sometime before the test. All of the isolates were tested by Sensi Disc (Nippon Becton Dickinson Co., Ltd., Tokyo), 23 isolates from case E and 11 isolates from case F, were resistant to tetracyline (TC); intermediately sensitive to streptomycin (SM); and sensitive to ampicillin (ABPC), cefotaxime (CTX), kanamycin (KM), gentamicin (GM), trimethoprim (TMP), ciplofloxacin (CPFX), fosfomycin (FOM), chloramphenicol (CP), sulfamethoxazole-trimethoprim (ST), and nalidixic acid (NA).

The 23 isolates from case E (Fig. 1 and 2) and two from case F (Fig. 2) were examined by pulsed-field gel electrophoresis (PFGE) using a Gene Path Typing System (Nippon Bio-Rad, Tokyo). The PFGE pattern of *Xba*I-digested chromosomal DNAs for the isolates from case E (Fig. 1 and lanes 9 and 10 in Fig. 2) was identical to that for case F (lanes 11 and 12 in Fig. 2); i.e., the two outbreaks were probably

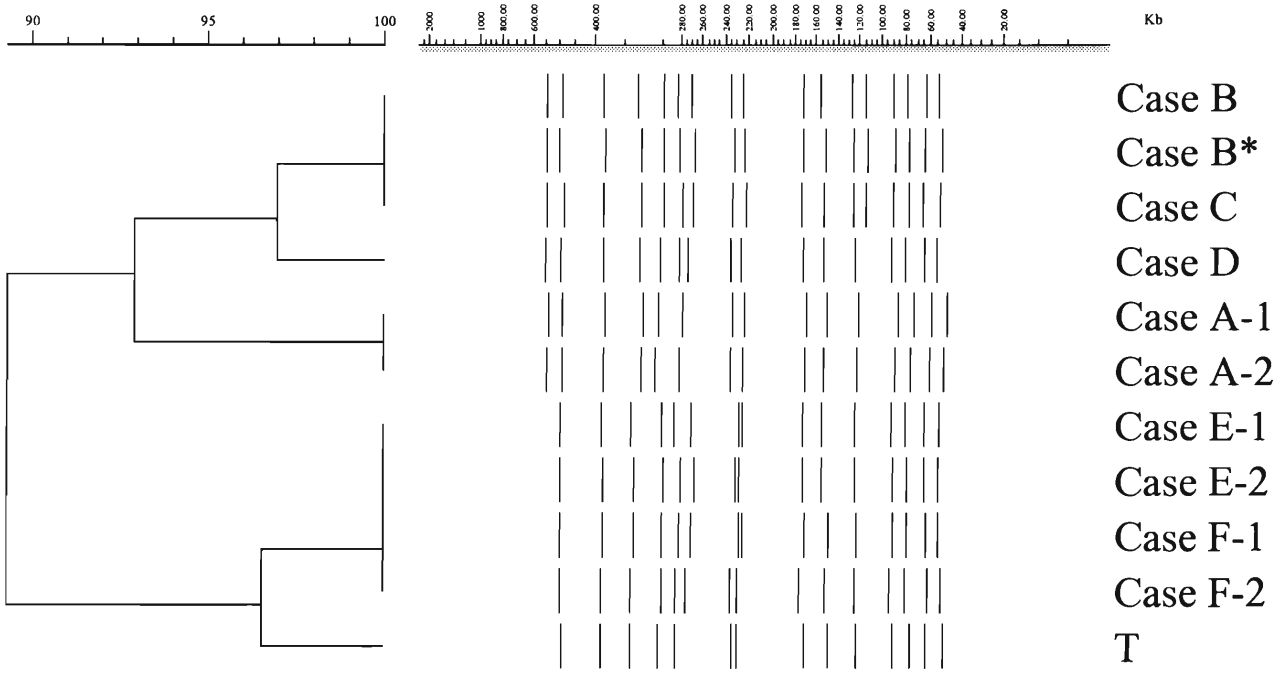

Fig.3. Cluster analysis of PFGE patterns of *E. coli* O169:H41 isolates shown in Fig. 2. The strains were all from patients, except in case B* (an isolate from *wakame*). T indicates an isolate from a traveler to India in 1999. Two isolates from the same outbreak are shown for cases A, E, and F.

caused by closely related strains. The pattern was compared with those of the strains from the four previous outbreaks in the Kinki district (case A, Takasago City and neighboring Towns in Hyogo Prefecture in June 1997; case B, Sakai City in Osaka Prefecture and Sumoto City in Hyogo Prefecture in April 1998; case C, Ohtsu City in Shiga Prefecture in June 1998; and case D, Seidan Town in Hyogo Prefecture in August 1998) (3) and that of an *E. coli* O169:H41 isolate from a traveler (T) infected in India in 1999 (Fig. 2). Isolates in cases A - F (A, lanes 2 and 3; B, lanes 5 and 6; C, lane 7; D, lane 4; E, lanes 9 and 10; and F, lanes 11 and 12) and an isolate in case T (lane 13) showed similar but slightly different PFGE patterns.

A dendrogram obtained by molecular analysis software (Finger Printing PLUS, Bio-Rad, Hercules, Calif., USA) (Fig. 3) indicated the presence of three distinct groups, designated as Group 1 (cases B, C, and D), Group 2 (case A), and Group 3 (cases E, F, and T). Thus, the outbreaks of STp-producing *E. coli* O169:H41 in the Kinki district over the last 3 - 4 years were caused by more or less genotypically different bacteria.

REFERENCES

1. Ando, K., Itaya, T., Aoki, A., Saito, A., Masaki, H. and Tokumara, Y. (1993): An outbreak of food poisoning caused by enterotoxigenic *Escherichia coli* O169:H41. Jpn. J. Food Microbiol.,10, 77-81 (in Japanese).
2. Nishikawa, Y., Hanaoka, M., Ogasawara, J., Moyer, N. P. and Kimura, T. (1995): Heat-stable Enterotoxin-producing *Escherichia coli* O169:H41 in Japan. Emerg. Infect. Dis.,1, 61.
3. Hamada, K., Tsuji, H. and Shimada, K. (1999): Outbreaks of heat stable enterotoxin-producing *Escherichia coli* O169 in the Kinki district in Japan: epidemiological analysis by pulsed-field gel electrophoresis. Jpn. J. Infect. Dis., 52, 165-167.
4. Macrina, F.L. (1984): Molecular cloning of bacterial antigens and virulence determinants. Ann. Rev. Microbiol., 38, 193-219.