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Outbreaks of Heat Stable Enterotoxin-Producing *Escherichia coli* O169 in the Kinki District in Japan: Epidemiological Analysis by Pulsed-Field Gel Electrophoresis

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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, four outbreaks due to the same organism occurred in Hyogo and neighboring Prefectures. Case A was identified in Takasago City in Hyogo Prefecture in June 1997. In April 1998, case B occurred in Sakai City in Osaka Prefecture and Sumoto City in Hyogo Prefecture. Case C was in Ohtsu City in Shiga Prefecture in June 1998, and case D was in Seidan Town in Hyogo Prefecture in August 1998 (Table 1 and Fig. 1). We obtained 50 isolates from the stool of symptomatic (diarrhea and/or abdominal pain) and asymptomatic patients. We isolated 3 strains from *wakame* seaweed implicated in outbreak B. In order to isolate the bacteria from *wakame*, the seaweed was first incubated in tetracycline-containing nutrient broth and then the broth was streaked on tetracycline-containing Lactose-MacConkey agar plates.

The isolates were examined for genes encoding LT, ST_h, ST_p, VT1, VT2, and InvE by polymerase chain reaction using primers purchased from Takara Shuzo Co. Ltd. (Kyoto). All 53 strains were negative for LT, ST_h, VT1, VT2, and InvE. Forty-nine strains were positive for the toxin gene ST_p but four were negative. The negative results might be explained by the curing of the Ent plasmid, which carries the toxin gene (3). All of the isolates were Tet resistant and NFLX sensitive; sensitivity to KM, SM, NA, and FOM was variable.

The isolates were examined by pulsed-field gel electrophoresis (PFGE) using the Gene Path Typing System (Nippon Bio-Rad, Tokyo). PFGE of *Xba*I digests gave two patterns (Fig. 2); samples from cases B and C had a heavy extra band (97-145.5 kb), which was absent in the samples derived from cases A and D. This observation indicated that cases B and C were caused by one strain and that cases A and D were caused by another. However, as the overall PFGE pattern was similar in both strains, they probably arose by mutation from a common

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Table 1. Outbreaks of *Escherichia coli* O169: Place, Implicated Food, Scale of Outbreak, and Isolated Microbes

Case	Place	Occasion ^a	Patients	Exposed ^b	Isolates (source) ^c
A	Takasago City and neighboring towns ^d	Box lunch	~2,800	~4,900	10 ST _P ++ (patients)
B	Sakai City Sumoto City	Lunch	762	2,035	7 ST _P ++ (patients)
		Lunch	35	53	22 ST _P ++ (patients) 2 ST _P ++ (<i>wakame</i>) 1 ST _P - (<i>wakame</i>)
C	Ohtsu City	Restaurant	43	73	6 ST _P ++ (patients) 2 ST _P - (patients)
D	Seidan Town	Dinner at an inn	56	133	2 ST _P ++ (patients)
					1 ST _P - (patient)

^aBox lunches or lunches were supplied by the same dealer in each case, one in Takasago City (case A) and one in Kobe City (case B). Implicated foods were known only for case B: *wakame* cooked with vinegar and *miso* (lunches for Sakai City) and cooked Japanese radish garnished with *wakame* (lunches for Sumoto City). The restaurant in case C is located at the Ohtsu Interchange of an expressway.

^bIndividuals who ingested the suspected food.

^cNumber of O169:H41 isolated. ST_P++ indicates a ST_P producer, and ST_P- a ST_P non-producer. Source of the isolates is indicated in the parentheses. All the isolates in case D were not determined for the H antigen. Seven isolates from Sakai City and 8 isolates from Ohtsu City were obtained from Shiga Prefectural Institute of Public Health and Environmental Science and Sakai City Institute of Public Health, respectively.

^dTakasago City, Himeji City, Kobe City, and the neighboring 4 cities and 3 towns.

Fig. 1. Location of outbreaks of *E. coli* O169:H41 in Hyogo and neighboring Prefectures.

strain. In large outbreaks of Shiga-toxin producing *E. coli* (STEC) in 1996 involving many locations in Japan (4,5), six types were detected, as distinguished by PFGE (4). In the STEC O157 outbreak in 1998 caused by contaminated *ikura* (salmon roe)-*sushi*, two different strains were isolated by PFGE (6).

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Fig. 2. PFGE patterns of *Xba*I digests of *E. coli* O169:H41 chromosomal DNAs.

Lane 1: λ DNA ladder; lanes 2-3: Isolates from patients in Sumoto City (case B); lane 4: isolates from *wakame* in Sumoto City (case B); lanes 5-7: isolates from patients in Sakai City (case B); lanes 8-10: isolates from patients in Takasago City (case A); lanes 11-13: isolates from patients in Ohtsu City (case C); lanes 14-15: isolates from patients in Seidan town (case D)

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