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# An Outbreak of Enterohemorrhagic *Escherichia coli* O157 Caused by Ingestion of Contaminated Beef at Grilled Meat-Restaurant Chain Stores in the Kinki District in Japan: Epidemiological Analysis by Pulsed-Field Gel Electrophoresis

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Enterohemorrhagic *Escherichia coli* (EHEC) is a highly virulent enteric pathogen. 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 (3-5).

Cattle are considered to be an important reservoir for EHEC, and so beef is one of the major causative foods for EHEC infection (1,6). From the end of April to the middle of May in 2002, an outbreak of the EHEC O157: H7 appearing first in Hyogo Prefecture and its boundary areas extended to Osaka, Kyoto, Nara, Wakayama, and Okayama Prefectures. The outbreak was due to contaminated raw beef materials procured by a restaurant center (in Himeji City in Hyogo) for its 63 branch restaurants which served grilled beef.

Investigations revealed 43 persons (1 to 77 years of age) who consumed beef at 19 such branch restaurants dispersed in Hyogo, Kyoto, Nara, Wakayama, and Okayama within a short range of time between April 26 and May 1 (except one person who ate beef on May 9). Twenty-eight among them developed enterocolitic symptoms several days later. The remaining 15 had no symptoms. We obtained a total of 23 isolates from their fecal specimens. In addition, we obtained four strains from suspected beef stocked in the central and three branch restaurants in Hyogo.

The strains were examined for genes encoding verotoxin (VT) using polymerase chain reaction employing EVT- (for VT1) and EVS- (for VT2) primers purchased from Takara Shuzo Co., Ltd., (Kyoto) (7). All the isolates were positive for both VT1 and VT2, except one isolate ('02-E.013; see lane 8 in Fig. 1B) which was positive for only VT1. Sensi Disk (Nippon Becton Dickinson Co., Ltd., Tokyo) assay (8)

revealed all the isolates were sensitive to all the 12 antibiotics: ampicillin, cefotaxime, kanamycin, gentamicin, streptomycin, tetracycline, trimethoprim, ciprofloxacin, fosfomycin, chloramphenicol, sulfamethoxazole-trimethoprim, and nalidixic acid.

Pulsed-field gel electrophoretic (PFGE) patterns of the 27 isolates were analyzed by using a gene path typing system (Program No. 5 or No. 27; Nippon Bio-Rad, Tokyo) in a manner reported previously (9). The typical PFGE patterns of *Xba*I-digested chromosomal DNAs are shown in Fig. 1. Five PFGE patterns were observed among human isolates and three patterns among beef isolates. Nineteen out of the 23 isolates from humans (lanes 1-7 in Fig. 1A and lanes 1-4, 5-6, 9-10, and 12 in Fig. 1B) and one out of four from food (lane 12 in Fig. 1A) gave an identical pattern, whereas four others from humans ('02-E.019 in lane 8 in Fig. 1A; '02-E.012 in lane 7, '02-E.013 in lane 8, and '02-E.015 in lane 11 in Fig. 1B) and three others from food (lanes 9-11 in Fig. 1A; PFGE patterns in lanes 10 and 11 were identical) were different from the predominant isolates from humans, e.g., '02-E.004 (lane 1 in Figs. 1A and 1B).

Three kinds of genetically different strains were isolated from four samples of beef, suggesting that beef contained a variety of EHECs. We speculate that patients were infected by strains predominant among the EHEC population present in the food. Probably 19 patients ate food contaminated by the dominant type '02-E.004, and at least two of the remaining four ate beef containing EHEC other than '02-E.004, such as '02-E.012 or '02-E.013. The origins of '02-E.012 and '02-E.013 were probably different from that of '02-E.004 because the PFGE patterns were largely different between the two groups. Meanwhile, we speculate that '02-E.015 and '02-E.019 were of the same origin as '02-E.004 and the change took place in a short period in infected humans owing to the high mutability of EHEC (10). This is because '02-E.015 and

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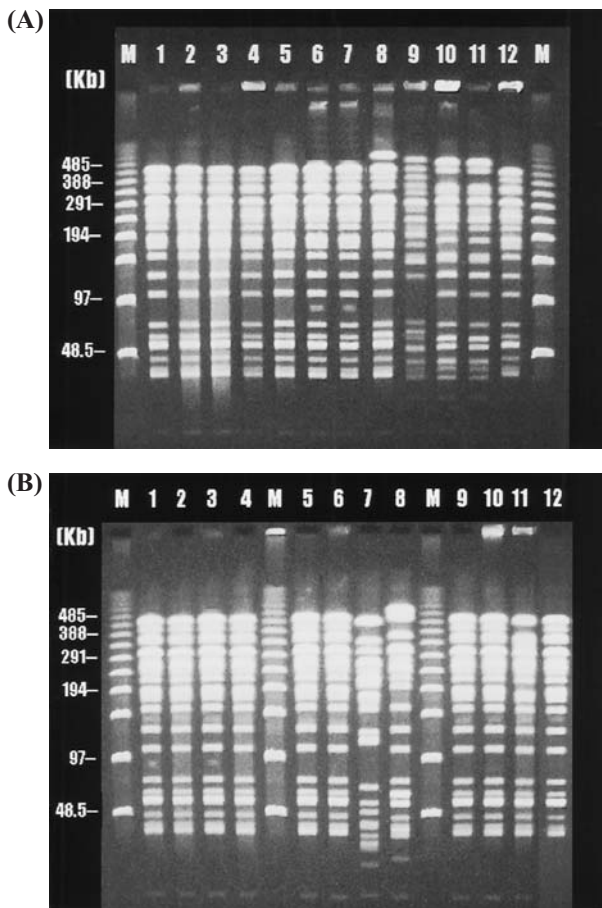


Fig. 1. PFGE patterns of *Xba*I-digests of chromosomal DNA of *Escherichia coli* O157 isolates. PFGEs were performed using Program No. 27. Otherwise the isolates from beef are noted in parentheses, and all the lane wells contain the isolates from human feces.

- (A) M:  $\lambda$ DNA ladder. Lane 1: '02-E.004. Lane 2: '02-E.032. Lane 3: '02-E.005. Lane 4: '02-E.031. Lane 5: '02-E.027. Lane 6: '02-E.028. Lane 7: '02-E.029. Lane 8: '02-E.019. Lane 9: '02-E.035 (from heart). Lane 10: '02-E.039 (from liver). Lane 11: '02-E.048 (from stringy beef). Lane 12: '02-E.044 (from rib).
- (B) M:  $\lambda$ DNA ladder. Lane 1: '02-E.004. Lane 2: '02-E.007. Lane 3: '02-E.008. Lane 4: '02-E.018. Lane 5: '02-E.010. Lane 6: '02-E.011. Lane 7: '02-E.012. Lane 8: '02-E.013. Lane 9: '02-E.014. Lane 10: '02-E.016. Lane 11: '02-E.015. Lane 12: '02-E.017.

'02-E.019 were only slightly different from '02-E.004 in PFGE, and especially because the patient from whom '02-E.015 was isolated and his brother from whom '02-E.014, identical to '02-E.004 in PFGE pattern (compare lanes 9 and 1 in Fig. 1B), was isolated ate beef together at the same restaurant.

The outbreak reported here was caused by a large amount of beef procured by a distribution center and supplied to branch restaurants. This epidemic is a typical diffuse outbreak. The epidemic demonstrates an inherent risk of this food delivery system of spreading food poisoning in a large geographical area.

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