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An Outbreak of Heat-Stable Enterotoxin-Producing *Escherichia coli* O25: HNM Resistant to Nalidixic Acid Caused by Ingestion of Contaminated Box-Lunch in September-October 2002 in Hyogo and Shiga Prefectures

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It has been considered in the late 1960s that enterotoxigenic *Escherichia coli* (ETEC) infections in Japan were primarily infections of overseas travelers. However, these infections have been increasingly identified among patients with diarrhea who had not traveled abroad. For the past two decades, approximately 800 ETEC strains have been isolated annually from Japanese patients, and about half of these patients had not traveled to foreign countries prior to their illness (1). *E. coli* serotypes O25: HNM (1) and O169: H41 (2,3) have been major causes of ETEC infections over the past decade in Japan. From September to October in 2002, Hyogo Prefecture (the end of Sept.) and a boundary area of Shiga and Fukui Prefectures (early Oct.) experienced an outbreak due to such ETECs showing nalidixic acid (NA)-resistance.

Among 1,838 persons belonging to 117 groups who ingested the suspected box-lunch as supplied by two delivery

companies in Cities A and B in Hyogo and Shiga Prefectures, respectively, 328 persons of 20-67 years of age developed symptoms such as diarrhea, fever, and abdominal pain. Two food materials, each produced by the same producer or manufacturer, were commonly used for the lunches prepared by the two companies. Of 119 patients and 34 employees working for the companies investigated, lysine-negative *E. coli* O25: HNM were isolated in stool specimens of 50 customers showing severe symptoms and in one employee. As a reference, we used one *E. coli* O25: HNM strain, '96-E.118, isolated from a sporadic infection in 1996 in one of our laboratories. The strain was lysine-negative, sensitive to all the drugs tested (see the below), and an STh enterotoxin producer.

The 51 strains from the outbreak 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 STp and positive for STh. The strains were tested for sensitivities to

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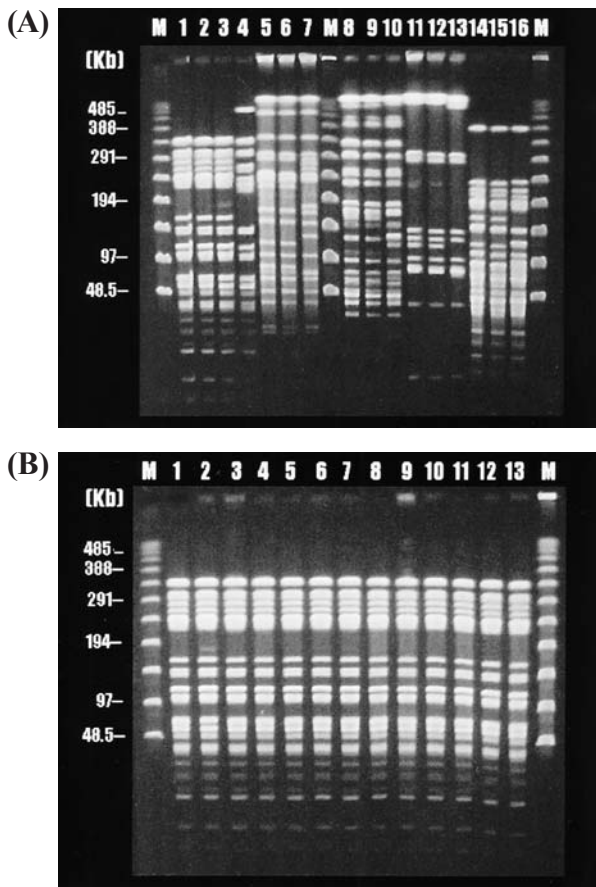


Fig. 1. PFGE patterns of digests with five kinds of restriction enzymes of chromosomal DNA of ETEC O25:HNM isolates. The PFGE was performed using a Gene Path Typing System, linear Program No. 5. (A) *XbaI*-, *NotI*-, *SfiI*-, *BlnI*-, or *SpeI*-digests of four ETEC O25:HNM isolates. The strains '02-E.098 (one of Hyogo strains), '02-E.142 (one of Shiga strains), and '02-E.143 (the same as '02-E.142) were isolated from this outbreak. The strain '96-E.118 was from a sporadic infection in 1996. Lanes 1-4: *XbaI*-, lanes 5-7: *NotI*-, lanes 8-10: *SfiI*-, lanes 11-13: *BlnI*-, and lanes 14-16: *SpeI*-digests. Lanes 1, 5, 8, 11, and 14: '02-E.142, lane 2: '02-E.143, lanes 3, 6, 9, 12, and 15: '02-E.098, and lanes 4, 7, 10, 13, and 16: '96-E.118. M: λ ladder. (B) *XbaI*-digests of 13 isolates from this outbreak. Lane 1: '02-E.098 and lanes 2-13: 12 strains, all of which were isolated in Hyogo Prefecture. M: λ ladder.

ampicillin, cefotaxime, kanamycin, gentamicin, streptomycin, tetracycline, trimethoprim, ciprofloxacin, fosfomycin, chloramphenicol, sulphamethoxazole- trimethoprim, and NA by using antibiotic disks (Becton Dickinson Microbiology Systems, Cockeysville, Md., USA)(4). All were resistant to NA and sensitive to the remaining drugs.

The pulsed-field gel electrophoresis (PFGE) patterns of DNA from ETEC O25 isolates were analyzed by using a Gene

Path Typing System (Program No. 5; Bio-Rad Laboratories, Hercules, Calif., USA). Bacterial chromosomal DNAs were digested with *XbaI*, *BlnI*, *SfiI*, *NotI*, or *SpeI* purchased from a manufacturer (Takara Shuzo). It was found that, of these digests, *XbaI*-digests gave the most suitable PFGE patterns for classifying the isolates (Fig. 1A). A pattern of 13 isolates from this food poisoning outbreak identical to the pattern of the *XbaI*-digests is shown in Fig. 1B. The strain from a sporadic infection ('96-E.118) gave a pattern different from those isolates with every digest (Fig. 1A).

The occurrences of the ETEC strains resistant to NA and fluoroquinolones have been rare up to the middle of the 1990s in Japan. Such strains started to increase abruptly from 1996 (5). The present case is probably the first reported case of a food poisoning outbreak caused by NA-resistant ETEC in Japan.

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