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Enterotoxigenic *Escherichia coli* O6:H16 Food Poisoning Outbreak in Prisons

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There was an outbreak of enterotoxigenic *Escherichia coli* (ETEC) on the same day (28 August 2005) in two facilities, a prison and a juvenile classification office (JCO), in Chiba City near Tokyo. The meal was prepared by the prison kitchen and transported by car to the JCO. Among 1,310 detainees in the two facilities, 401 developed symptoms, such as watery diarrhea (95%) and abdominal pain (80%); symptoms were generally mild.

Bacteriological investigation was made on 11 swab specimens, 49 preserved meal specimens, 2 specimens of unprepared material (celery cabbage and kimuchi essence) and 93 stool specimens of patients (including 6 cooks). LT/ST-producing ETEC O6:H16 was isolated from the preserved celery cabbage kimuchi and 81 stool specimens (Table 1). The cooks whose stool specimens were ETEC-positive were detainees performing the job on rotation and were healthy before the outbreak.

The methods of detection and identification of ETEC were as follows. The colonies on the DHL agar (Pearl Core; Eiken Chemical, Tokyo, Japan) were submitted to colony-sweep PCR (1), and when positive for LT or ST gene (2), they were further submitted to screening of toxin production (VET-RPLA Seiken and Colist EIA Seiken; Denka Seiken Co., Tokyo, Japan) and to serotyping (*Escherichia coli* Antisera Seiken; Denka Seiken).

For amplification of *E. coli*, we inoculated mEC Broth (supplemented with Novobiocin; Eiken Chemical) with foods that had been conserved at 4°C. The mEC broth culture was then used for colony isolation on DHL agars. The colony-sweep PCR detected LT and ST genes from the culture of the preserved kimuchi. However, as *Klebsiella* colonies dominated the plates, we were unable to identify *E. coli* colonies. Therefore, we thoroughly mixed 1 ml of 10% homogenate of the frozen preserved foods and 15 ml of desoxycholate agar medium (kept at 50°C) in a Petri dish; then, after solidification of the agar, the cultures were incubated at 37°C for colony development. Among 200 colonies that developed, 50 colonies were picked and plated onto BTB Lactose agar (Nissui, Tokyo, Japan) and EMB agar (Eiken Chemical). One colony turned positive for ETEC.

Table 1. Detection of ETEC O6:H16

Specimen	No. of specimens	No. of positives
Swabs	11	0
Preserved foods	49	1
Unprepared materials of kimuchi	2	0
Stool specimens		
Detainees	69	58
Cooks	6	6
Detainees in JCO	18	17
Total	155	82

JCO, juvenile classification office.

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The 18 isolated ETEC strains (1 from kimuchi, 11 from patients and 6 from cooks) were submitted to pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA. All the isolates were identical in PFGE (Fig. 1) and drug sensitivity patterns (all were sensitive to 12 antibiotics tested, i.e., streptomycin, ampicillin, trimethoprim, fosfomycin, trimethoprim-sulphamethoxazole, gentamicin, tetracycline, norfloxacin, nalidixic acid, kanamycin, cefotaxime, chloramphenicol).

The above data indicated that the outbreak was caused by the contaminated celery cabbage kimuchi served as lunch on 27 August. However, we could not isolate ETEC from unprocessed celery cabbage and kimuchi essence, which were used for the preparation of the kimuchi.

Colony-sweep PCR was found a powerful screening method for detecting ETEC from the stool and food specimens. However, it required much time and manpower. As ETEC-specific selection media are unavailable, we had to screen large numbers of *E. coli*-like colonies.

Our rough calculation indicated that the number of ETEC bacilli in the preserved kimuchi was 10 - 40 bacteria/g. As it

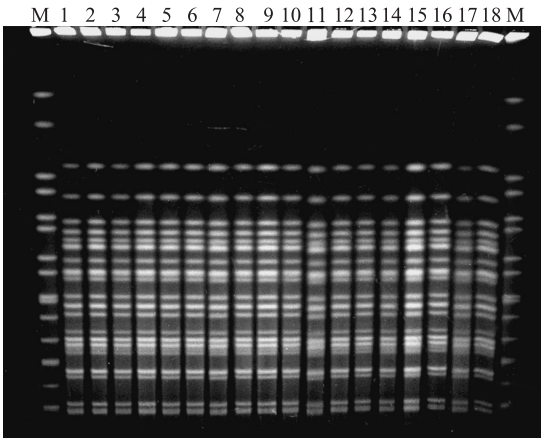

Fig. 1. PFGE pattern of *Xba*I-digested chromosomal DNA of ETEC isolates. Lane 1, isolate from kimuchi; lanes 2-7, isolates from the cooks; lanes 8-18, isolates from patients in the prison and JCO; M, *Salmonella* Braenderup H9812 strain.

was left for 3 h at the room temperature (about 35°C) before freezing, bacteria must have grown during the incubation period. Therefore, the number of bacteria in the kimuchi ingested by the patients should have been lower than that number. Assuming that the consumed amount of kimuchi per person was 25 g per person and the kimuchi contained 10 - 40 bacteria/g, we calculated that the number of bacteria each person ingested was 100 - 1,000. Considering the growth of bacteria during the 3-h-incubation period before freeze, the ingested bacterial number could have been even lower. Therefore, it was suggested that a very small number of bacteria could produce the symptomatic infection.

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REFERENCES

1. Konishi, N., Obata, H., Shimojima, Y., Shibata, M., Akiba, T., Monma, C., Kai, A. and Morozumi, S. (2004): An outbreak of food intoxication caused by lunch boxes provided by a caterer, from which six different types of enterotoxigenic *Escherichia coli* were isolated: methods of detection and the results of investigation. Proceedings of the 16th Japanese Prefectural Institutes of Public Health, Kanto-Koshinsei-Branch, Committee on Bacteriology (in Japanese).
2. Ito, F., Ogino, T., Ito, K. and Watanabe, H. (1992): Simultaneous detection of multiple pathogenic genes of diarrhea-causing *Escherichia coli* with PCR by using primer mixtures. Jpn. J. Clin. Med., 50 (Suppl.), 343-347 (in Japanese).