Laboratory and Epidemiology Communications

A Food Poisoning Diarrhea Outbreak Caused by Enteroaggregative
*Escherichia coli* Serogroup O126:H27 in Shizuoka, Japan

Tetsuya Harada*, Midori Hiroi, Fumihiko Kawamori, Aki Furusawa, Katsuhiro Ohata, Kanji Sugiyama and Takashi Masuda

Shizuoka Institute of Environment and Hygiene, Shizuoka 420-8637, Japan

Communicated by Haruo Watanabe

(Accepted April 3, 2007)

Enteroaggregative *Escherichia coli* (EAEC), which is identified by a special characteristic “stacked brick” aggregative adherence to cultured human epithelial cells (1), has been associated with acute and persistent diarrhea in children, in food poisoning diarrhea outbreaks and in overseas travelers (2). In Japan, a massive food-borne outbreak of gastrointestinal illness caused by EAEC O untypeable:H11 occurred in Gifu Prefecture (3) and a diarrheal outbreak associated with EAEC O126:NM was reported in Akita Prefecture (4).

On August 22-23, 2005, a diarrhea outbreak due to food poisoning occurred in a police institute in M City in Shizuoka Prefecture. Of 9 members admitted to the institute, 7 showed clinical symptoms, including diarrhea (in all patients) and fever (2 patients). Fecal samples were obtained from 5 patients on 27 August and 2 food handlers on 29 August and were subjected to examination for diarrheagenic pathogens. *E. coli* O126:H27 was isolated from 4 patients and one food handler. A multiplex polymerase chain reaction (PCR) assay was performed with the specific pair primers described previously for the identification of the following diarrheagenic *E. coli* virulence genes: *eae* (5), *bfp* (6), *elt* (7), *estA* (8), *sts1* (9), *vt-2* (10), *ipaH* (11) and *astA* (12). 297pCVD432-F (5´-CGG CTT ATG AAG CAA AAA TGC-3´) and 297pCVD432-R (5´-CCT CCT CCT CAA GGA CAT CA-3´) primers were also designed from published *E. coli* plasmid pCVD432 DNA sequences (DDBJ RELEASE: X81423) to determine the presence of a 297-bp fragment for an EAEC CVD432 probe (13). The amplification conditions were as follows: initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 45 sec, annealing at 60°C for 45 sec and extension at 72°C for 1 min, followed by an elongation step at 72°C for 5 min. The multiplex PCR assay showed that all 5 *E. coli* O126:H27 isolates gave positive results for *astA* and the EAEC CVD432 probe (data not shown). Subtyping of these 5 strains was performed by pulsed-field gel electrophoresis (PFGE) with XbaI-digested DNA using a CHEF-DR III apparatus (Nippon Bio Rad Laboratories, Tokyo, Japan) and the PFGE patterns were found to show complete agreement (Fig. 1). In the adhesion assay, the two *E. coli* O126:H27 strains isolated from one patient and one food handler showed aggregative adherence patterns to HEp-2 cells (data not shown). These results suggest that EAEC O126:H27 was the causative pathogen of this food poisoning diarrhea outbreak.

Moreover, XbaI-restricted PFGE analysis using the Fingerprinting II software (version 3.0, Nippon Bio Rad) was carried out to study the genetic relatedness among *E. coli* O126 strains harboring *astA*, *aggR* and the EAEC CVD432 probe in Shizuoka Prefecture. The PFGE profiles of EAEC

*Corresponding author: Mailing address: Shizuoka Institute of Environment and Hygiene, 4-27-2 Kita-ando, Aoi-ku, Shizuoka 420-8637, Japan. Tel: +81-54-245-0291, Fax: +81-54-245-7636, E-mail: tetsuya1_harada@pref.shizuoka.lg.jp
O126:H27 from a patient in this food poisoning diarrhea outbreak and 3 E. coli O126:H27 and 2 E. coli O126:H untypeable strains recovered from sporadic diarrhea cases from April 2004 to March 2006 showed a similarity of >80% (Fig. 2), suggesting that genetically close E. coli O126 strains with specific gene profiles may have a wide distribution in Shizuoka Prefecture.

REFERENCES