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An Outbreak of Food-Borne Gastroenteritis Caused by *Clostridium perfringens* Carrying the *cpe* Gene on a Plasmid

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Clostridium perfringens enterotoxin (CPE) is an important virulence factor for *C. perfringens* type A food poisoning. The *cpe* gene was present on a chromosome in food poisoning isolates but on a plasmid in non-food-borne human gastrointestinal disease isolates (1,2). Here we describe an outbreak of food-borne gastroenteritis caused by *C. perfringens* isolates carrying a plasmid-borne *cpe* gene.

On 31 May 2001, an outbreak of gastroenteritis occurred in a nursing home for the aged in Toyama Prefecture, Japan. Of 192 persons who ate a lunch prepared at a catering facility, 90 became ill. The predominant symptoms were diarrhea (99%) and abdominal pain (41%). The mean incubation period was 15.5 h.

A total of 107 stool specimens collected on 1 and 2 June were examined bacteriologically. Stool cultures were negative for *Salmonella*, *Vibrio*, *Campylobacter*, and *Bacillus*. However, *C. perfringens* was cultured from 90 samples. Twenty-four isolates selected randomly were examined for CPE production by using modified DS (mDS) medium (3) and PET-RPLA (Denka Seiken, Tokyo); 22 isolates (92%) were shown to be CPE positive. Pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion was performed for eight of these 22 CPE-positive isolates (Fig. 1). The eight isolates showed identical PFGE patterns. These eight isolates were serotype TW54.

Boiled bean was the most highly suspected vehicle because it was cooked in large quantities, cooled slowly after cooking, and served without adequate reheating. However, no boiled beans remained. Specimens of seven other foods remaining from lunch and 13 environmental swabs were all negative for *C. perfringens*.

PFGE Southern blot analysis was performed to establish the chromosomal or plasmid location of the *cpe* gene, following a procedure previously described (4-6). The principle of this method was as follows. *C. perfringens* chromosomal DNA is too large to enter a pulsed-field gel without any restriction enzyme digestion, whereas most plasmid DNA enters a

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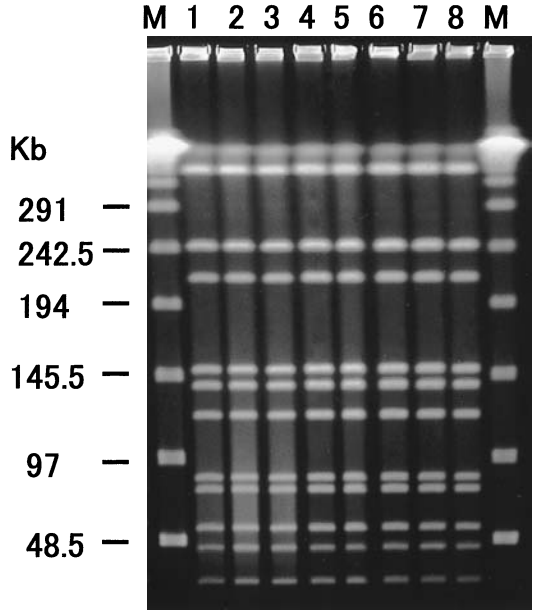

Fig. 1. PFGE patterns of *Sma*I-digested chromosomal DNA of *C. perfringens* isolates. M: λDNA ladder, Lanes 1-8: isolates from different patients. The gel electrophoresis was performed with CHEF-DRIII (BioRad, Hercules, Calif., USA) under the following conditions: voltage, 6 V/cm; pulse time, 5-20 s within 20 h; buffer, 0.5× TBE buffer with 50 μM thiourea.

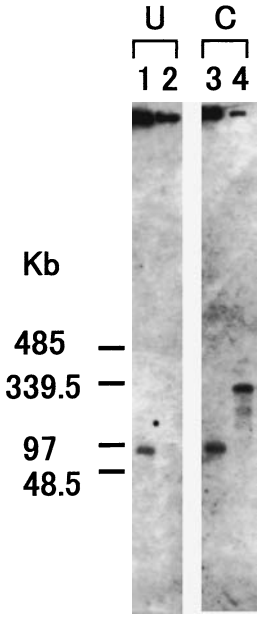

Fig. 2. PFGE Southern blot analysis of *C. perfringens* isolates. PFGE and Southern hybridization analysis of undigested (U) and I-*Ceu*I cut (C) DNA from selected isolates. Southern hybridization analysis was performed with the AlkPhos Direct Labelling and Detection System (Amersham Pharmacia Biotech, Buckinghamshire, UK) using a 639-bp *cpe* probe. Lanes 1 and 3: isolate T102 from a patient food-poisoned in the recent outbreak, Lanes 2 and 4: strain Osaka4205.

pulsed-field gel without any restriction enzyme digestion. In addition, since I-*Ceu*I sites are located exclusively on the *C. perfringens* chromosome but not on the plasmid, digestion of DNA samples with I-*Ceu*I produces chromosomal DNA fragments that can enter pulsed-field gels but does not affect the migration of plasmid DNA. We discovered that isolate T102 from this outbreak carried a plasmid *cpe* (Fig. 2). Similar PFGE Southern blot results were obtained with two isolates from different patients (data not shown). In contrast, strain Osaka4205 from other food poisoning outbreaks carried a chromosomal *cpe* gene.

The heat sensitivities of spores produced by *C. perfringens* isolates were examined. Three isolates from this food poisoning outbreak and strain Osaka4205 from another food poisoning outbreak were cultured anaerobically overnight at 37°C in mDS medium. One milliliter of each mDS culture was heated at 80 and 100°C, respectively, for 10 min. A 0.1-ml aliquot of each mDS medium culture was then inoculated into 10 ml of cooked meat medium (Difco, Detroit, Mich., USA), incubated overnight at 37°C, then examined for growth. The spores of the three isolates from this food poisoning outbreak showed resistance to heating at 80°C for 10 min, but not at 100°C for 10 min. In contrast, strain Osaka4205 showed resistance to heating at both 80°C for 10 min and 100°C for 10 min. Therefore, the present food poisoning isolates were thought to be the heat-sensitive spore-forming *C. perfringens*. This finding is in agreement with that of Sarker et al. (6), who reported that the spores of chromosomal *cpe* isolates were more heat resistant than those plasmid *cpe* isolates. Tórtora et al. (7) reported that enterotoxigenic heat-resistant spore-forming *C. perfringens* isolates could not ferment trehalose and inositol, whereas enterotoxigenic heat-sensitive spore-forming *C. perfringens* isolates were trehalose- and inositol-fermentative. The food poisoning isolates presented here fermented these two sugars. To our knowledge, this is the first case report of a food-borne disease outbreak caused by *C. perfringens* isolates carrying a plasmid *cpe* gene.

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