

## Laboratory and Epidemiology Communications

### Genotyping of *Clostridium perfringens* Isolates Collected from Food Poisoning Outbreaks and Healthy Individuals in Japan Based on the *cpe* Locus

Daisuke Tanaka\*, Keiko Kimata, Miwako Shimizu, Junko Isobe, Masanori Watahiki, Tadahiro Karasawa<sup>1</sup>, Takayoshi Yamagishi<sup>1</sup>, Sanae Kuramoto<sup>2</sup>, Toshihiko Serikawa<sup>2</sup>, Fubito Ishiguro<sup>3</sup>, Makiko Yamada<sup>4</sup>, Kazukiyo Yamaoka<sup>4</sup>, Mitsuo Tokoro<sup>4</sup>, Toshio Fukao<sup>5</sup>, Masakado Matsumoto<sup>6</sup>, Reiji Hiramatsu<sup>6</sup>, Chie Monma<sup>7</sup> and Yoshiyuki Nagai

*Toyama Institute of Health, Toyama 939-0363; <sup>1</sup>Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, Ishikawa 920-0942; <sup>2</sup>Ishikawa Prefectural Institute of Public Health and Environmental Science, Ishikawa 920-1154; <sup>3</sup>Fukui Prefectural Institute of Public Health and Environmental Science, Fukui 910-8551; <sup>4</sup>Gifu Prefectural Institute of Health and Environmental Sciences, Gifu 504-0838; <sup>5</sup>Gifu Municipal Institute of Public Health, Gifu 500-8881; <sup>6</sup>Aichi Prefectural Institute of Public Health, Aichi 462-8576; and <sup>7</sup>Tokyo Metropolitan Research Laboratory of Public Health, Tokyo 169-0073, Japan*

Communicated by Yoshichika Arakawa

(Accepted December 25, 2006)

*Clostridium perfringens* type A isolates carrying the enterotoxin gene (*cpe*) are responsible for both food poisoning and non-foodborne human diseases such as antibiotic-associated diarrhea and sporadic diarrhea. In North America and Europe, food poisoning isolates were found to carry a chromosomal *cpe* gene, while non-foodborne gastrointestinal disease isolates were shown to have a plasmid *cpe* gene (1-3). However, we reported that *C. perfringens* isolates collected from three food poisoning outbreaks carried the *cpe* gene on a plasmid (4,5). Recently, Miyamoto et al. (6) developed multiplex PCR *cpe* genotyping that is able to distinguish among *C. perfringens* type A isolates carrying either a chromosomal *cpe* gene, a plasmid *cpe* gene with a downstream IS1470-like sequence, or a plasmid *cpe* gene with a downstream IS1151 sequence. In an attempt to clarify the epidemiology of *cpe*-positive *C. perfringens* isolates collected from foodborne outbreaks and healthy individuals in Japan, we subjected 48 *cpe*-positive isolates to multiplex PCR *cpe* genotyping. Of these, 31 isolates were collected from patients involved in 13 unrelated

food poisoning outbreaks, 16 isolates were from the feces of healthy individuals, and 1 isolate was collected from the environment during a non-foodborne disease outbreak.

As shown in Table 1, all 31 *C. perfringens* isolates collected from food poisoning outbreaks were successfully typed by multiplex PCR *cpe* genotyping: 15 isolates from eight food poisoning outbreaks carried the chromosomal *cpe* gene, 10 isolates from four food poisoning outbreaks carried the plasmid *cpe* gene with a downstream IS1151 sequence, and 6 isolates from two food poisoning outbreaks carried the plasmid *cpe* gene with a downstream IS1470-like sequence. The food-poisoning isolates collected from Fukui Prefecture included both chromosomal *cpe* isolates and plasmid *cpe* isolates. These results suggest that *C. perfringens* food poisoning outbreaks have frequently been caused by plasmid *cpe* isolates, and isolates carrying the plasmid *cpe* gene with a downstream IS1151 sequence appear to predominate. However, all three mass food poisoning outbreaks with >400 patients were caused by chromosomal *cpe* isolates. These observations are consistent with those of a previous study (6) that chromosomal *cpe* isolates are a common cause of typical *C. perfringens* food poisoning outbreaks. However, *C. perfringens* carrying a plasmid *cpe* gene with a downstream IS1151 sequence was present in the *cpe*-positive isolates

\*Corresponding author: Mailing address: Department of Environmental Biology and Chemistry, Faculty of Science, University of Toyama, Toyama 930-8555, Japan. Tel: +81-76-445-6673, Fax: +81-76-445-6549, E-mail: tanakada@sci.u-toyama.ac.jp

Table 1. List of *C. perfringens* isolates obtained from food-borne outbreaks in Japan and location of *cpe* locus

Year/month	Place (Prefecture)	Setting	No. of patients	Vehicle	Serotype	No. of isolates	<i>cpe</i> locus
1968/10	Shizuoka	Business establishment	21	Catering lunch, meatball	TW6	1	Chromosome
1980/3	Toyama	Hospital	61	Breakfast, including crab salad and egg soup with spinach	TW46	3	Plasmid (IS1470-like)
1998/12	Aichi	Business establishment	401	Catering lunch, curry with macaroni	TW64	3	Chromosome
1999/9	Gifu	Travel group	74	Unknown	Hobbs 5	3	Plasmid (IS1470-like)
2001/5	Toyama	Nursing home	90	Catering lunch, boiled bean (most probably)	TW54	3	Plasmid (IS1151)
2002/7	Gifu	Restaurant	78	Party food	TW62	3	Plasmid (IS1151)
2002/11	Toyama	Restaurant	687	Catering lunch, stew	TW24/26	3	Chromosome
2002/11	Ishikawa	Lunch provider	540	Box lunch	Hobbs 4	1	Chromosome
					Hobbs 16	1	Chromosome
2003/4	Toyama	Nursing home	67	Breakfast	TW54	3	Plasmid (IS1151)
2003/6	Aichi	Business establishment	70	Bean curd topped with a sticky sauce	TW64	2	Chromosome
2003/7	Gifu	Restaurant	48	Vegetables boiled and seasoned (most probably)	Hobbs 13	1	Chromosome
2004/3	Fukui	Nursing home	22	Parched soybean fiber (most probably)	TW38	1	Chromosome
					TW12	1	Plasmid (IS1151)
					TW24/64	1	Chromosome
2004/8	Gifu	Hospital	56	Providing meals	Hobbs 1	1	Chromosome

collected from three food poisoning outbreaks occurring in nursing homes. Moreover, one *C. perfringens* isolate collected from a non-foodborne diarrhea outbreak in a nursing home (7) also carried the plasmid *cpe* gene with a downstream IS1151 sequence (data not shown). It is possible that *C. perfringens* isolates carrying the plasmid *cpe* gene with a downstream IS1151 sequence have been responsible for foodborne and non-foodborne outbreaks in nursing homes. With regard to the serotype of *C. perfringens* isolates collected from foodborne outbreaks, a wide variety of serotypes were observed. Five isolates that reacted with TW64 serum carried the chromosomal *cpe* gene, and 6 isolates that reacted with TW54 carried the plasmid *cpe* gene with a downstream IS1151 sequence.

We also examined *cpe*-positive *C. perfringens* isolates from healthy individuals (data not shown). Of the 16 isolates collected from healthy individuals, 11 carried a plasmid *cpe* gene with a downstream IS1151 sequence, 3 carried a plasmid *cpe* gene with a downstream IS1470-like sequence, and 2 carried a chromosomal *cpe* gene. Therefore, plasmid *cpe* isolates collected from healthy individuals and foodborne outbreaks commonly contain an IS1151 sequence downstream of the plasmid *cpe* gene. A previous study (6) suggested that the presence of downstream IS1470-like or IS1151 sequences in plasmid *cpe* isolates from non-foodborne gastrointestinal disease specimens in the United Kingdom was nearly equal; however, the isolates collected in North America commonly showed the presence of a downstream IS1470-like sequence. It is possible that geographic variations exist in *C. perfringens* carrying a plasmid *cpe* gene. In conclusion, we found that in Japan, *C. perfringens* food poisoning outbreaks may frequently be caused by plasmid *cpe* isolates present in a consistent abundance ratio among healthy individuals. Moreover, among the plasmid *cpe* isolates collected from both food

poisoning outbreaks and healthy individuals, *C. perfringens* carrying a plasmid *cpe* gene with a downstream IS1151 sequence appears to predominate.

We gratefully acknowledge Dr. Kazuaki Miyamoto (Department of Microbiology, Wakayama Medical College, Wakayama, Japan) for his advice on multiplex PCR *cpe* genotyping.

## REFERENCES

- Collie, R.E. and McClane, B.A. (1998): Evidence that the enterotoxin gene can be episomal in *Clostridium perfringens* isolates associated with non-food-borne human gastrointestinal diseases. *J. Clin. Microbiol.*, 36, 30-36.
- Cornillot, E., Saint-Joanis, B., Daube, G., et al. (1995): The enterotoxin gene (*cpe*) of *Clostridium perfringens* can be chromosomal or plasmid-borne. *Mol. Microbiol.*, 15, 639-647.
- Sparks, S.G., Carman, R.J., Sarker, M.R., et al. (2001): Genotyping of enterotoxigenic *Clostridium perfringens* fecal isolates associated with antibiotic-associated diarrhea and food poisoning in North America. *J. Clin. Microbiol.*, 39, 883-888.
- Tanaka, D., Isobe, J., Hosorogi, S., et al. (2003): An outbreak of food-borne gastroenteritis caused by *Clostridium perfringens* carrying the *cpe* gene on a plasmid. *Jpn. J. Infect. Dis.*, 56, 137-139.
- Nakamura, M., Kato, A., Tanaka, D., et al. (2004): PCR identification of the plasmid-borne enterotoxin gene (*cpe*) in *Clostridium perfringens* strains isolated from food poisoning outbreaks. *Int. J. Med. Microbiol.*, 294, 261-265.
- Miyamoto, K., Wen, Q. and McClane, B.A. (2004): Multiplex PCR genotyping assay that distinguishes between isolates of *Clostridium perfringens* type A carrying a chromosomal enterotoxin gene (*cpe*) locus, a plasmid *cpe* locus with an IS1470-like sequence, or a plasmid *cpe* locus with an IS1151 sequence. *J. Clin. Microbiol.*, 42, 1552-1558.
- Fukao, T., Sato, M., Tanaka, Y., et al. (2004): An outbreak of diarrheal disease caused by enterotoxigenic *Clostridium perfringens* following exposure to a contaminated environment in a nursing home. *J. Jpn. Assoc. Infect. Dis.*, 78, 32-39 (in Japanese).