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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 Karasawa1, Takayoshi Yamagishi1, Sanae Kuramoto2, Toshihiko Serikawa2, Fubito Ishiguro3, Makiko Yamada4, Kazukiyo Yamaoka4, Mitsuaki Tokoro4, Toshiaki Fukao5, Masakado Matsumoto6, Reiji Hiramatsu6, Chie Monma7 and Yoshiyuki Nagai

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

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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 IS1151-like sequence, or a plasmid cpe gene with a downstream IS1470-like 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.
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.

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### REFERENCES