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**Clostridium perfringens** Type A Strains Carrying a Plasmid-Borne Enterotoxin Gene (Genotype IS1151-cpe or IS1470-like-cpe) as a Common Cause of Food Poisoning

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The prevalences of various genotypes of enterotoxin gene-carrying (cpe-positive) **Clostridium perfringens** type A in 24 different food poisoning outbreaks were 75% (chromosomal IS1470-cpe), 21% (plasmid-borne IS1470-like-cpe), and 4% (plasmid-borne IS1151-cpe). These results show that **C. perfringens** type A carrying the plasmid-borne cpe is a common cause of food poisoning.

**Clostridium perfringens** strains are classified into five types (A to E) based on their expression of alpha, beta, epsilon, and iota toxins. Enterotoxin gene-carrying (cpe-positive) **C. perfringens** type A is a common cause of food poisoning and is also involved in sporadic and antibiotic-associated diarrhea (19). Fewer than 5% of **C. perfringens** strains carry cpe (9, 22).

The cpe-positive **C. perfringens** type A strains are divided into various genotypes based on the insertion sequence elements attached to cpe. IS1470 is found downstream of the chromosomal cpe (1), whereas either IS1470-like or IS1151 is located downstream of the plasmid-borne cpe (3, 14). The chromosomal cpe-carrying **C. perfringens** strains are associated with food poisoning, whereas the plasmid-borne cpe is typical for **C. perfringens** strains isolated from sporadic or antibiotic-associated diarrhea as well as those of veterinary origin (20).

The **C. perfringens** strains that carry the chromosomal cpe are more resistant to heating, osmotic stress, and low temperatures than are the plasmid-borne, cpe-carrying strains (11, 12). These resistances may explain the presence of chromosomal cpe-carrying strains in retail foods (23) and the predominance of these strains in food poisoning (1, 3, 8, 11, 16, 18, 20, 23). The plasmid-borne cpe-carrying strains are thus considered atypical causes of **C. perfringens** type A food poisoning (16, 21).

In the present study, the involvement of various genotypes of cpe-positive **C. perfringens** type A in food poisonings was investigated by examining a collection of 53 **C. perfringens** isolates, 26 from patients and 27 from various foods associated with 11 Finnish and 13 German food poisoning outbreaks from 1984 to 2007 (Table 1). DNA was isolated as described previously by Hytiä et al. (7) or was isolated with Advamax beads (Edge BioSystems, Gaithersburg, MD) according to the instructions of the manufacturer. Multiplex PCR was used to determine the toxinotype and presence of cpe (5). We studied the cpe genotype (IS1151-cpe, IS1470-like-cpe, or IS1470-cpe) of the cpe-positive isolates by detecting different insertion sequence elements downstream of cpe by using PCR with the previously described primers (1, 2, 4, 14, 15) and protocols (6).

The cpe-positive isolates were sporulated in modified Duncan and Strong medium (Sigma-Aldrich Chemie, Steinheim, Switzerland) as described previously by Miwa et al. (13). Successful sporulation was verified with a phase-contrast microscope, and **C. perfringens** enterotoxin (CPE) production was analyzed by reverse passive latex agglutination (RPLA) (PET-RPLA kit; Oxoid Ltd., Basingstoke, United Kingdom), according to the instructions of the manufacturer. cpe-positive and cpe-negative strains were used as controls in PCR assays and PET-RPLA.

In pulsed-field gel electrophoresis (PFGE) analysis, DNA was digested with ApaI and SmaI (New England Biolabs, Beverly, MA) and the genetic relationships between isolates were assessed using the previously described assay (17), which was modified by adding thioourea to the electrophoresis running buffer (10). The PFGE patterns were analyzed visually.

All isolates were of type A, and 48 (91%) carried cpe. The chromosomal IS1470-cpe was detected in 8 (73%) of the Finnish and 10 (77%) of the German outbreaks. The plasmid-borne IS1470-like-cpe was detected in three (27%) of the Finnish and in five (21%) of the German outbreaks, whereas the plasmid-borne IS1151-cpe was detected only in one (8%) of the German outbreaks. The prevalences in the Finnish and German **C. perfringens** outbreaks together were 75% for the chromosomal IS1470-cpe, 21% for the plasmid-borne IS1470-like-cpe, and 4% for the plasmid-borne IS1151-cpe (Table 1). Sporulation was successful, with 34 (70%) of the isolates investigated, and all of these isolates produced CPE (Table 1). A total of 31 different PFGE patterns were observed (Table 1). The finding of plasmid-borne, cpe-carrying genotypes presenting a notable proportion (25%) of outbreak strains differs from the usual assumption that chromosomal cpe-carrying strains are responsible for almost all **C. perfringens** food poisoning outbreaks. It is also interesting that the strains carrying plasmid-borne IS1470-like-cpe were considerably more common than the strains carrying plasmid-borne IS1151-cpe. Further studies are warranted to elucidate whether the phenomenon holds true globally and which factors contribute to these.
differences in the prevalence. To our knowledge, this is the first time that plasmid-borne cpe-carrying isolates indistinguishable by PFGE were found in both food and patient feces in a food poisoning outbreak caused by cpe-positive C. perfringens type A (Table 1). Since plasmid-borne, cpe-carrying strains are a common cause of food poisoning, some of the sporadic diarrheas caused by C. perfringens type A may also be food borne.

It was proposed that meat dishes may be typical vehicles for chromosomal cpe-carrying strains, whereas plasmid-borne cpe-carrying strains may cause food poisoning by atypical vehicles
such as vegetarian foods (13). This theory is not supported by our results, since strains carrying both chromosomal and plasmid-borne cpe were found in all types of food. However, a topic worthy of speculation could be the step in the food-handling process at which the food is contaminated with strains possessing different cpe-positive genotypes. Chromosomal cpe-carrying strains are often present in retail foods (23) and are more resistant to most food-processing conditions, such as heating, low temperatures, and high-salt concentrations, than plasmid-borne cpe-carrying strains (11, 12). These resistances could indicate that foods may be contaminated with chromosomal cpe-carrying strains at any step of the food-handling process, whereas contamination with plasmid-borne, cpe-carrying strains may occur at later stages, subsequent to storage and heating. Humans are a reservoir for different genotypes of cpe-positive C. perfringens type A, and thus, the food may become contaminated at any stage of the processing by the person handling the food (6). Foods that need handling during preparation thus may be more susceptible to contamination by humans. Interestingly, in outbreaks 2, 5, and 15, cpe-positive isolates with markedly different PFGE patterns were isolated within the same outbreak and in outbreak 15 even from the same food (Table 1). The isolates represented both chromosomal and plasmid-borne cpe-carrying genotypes, and PET-RPLA showed that all these strains were capable of producing CPE. Finding unrelated cpe-positive strains in the same outbreak indicates that food may be contaminated with multiple cpe-positive strains, leading to the growth of these strains under optimal conditions.

In conclusion, plasmid-borne cpe-carrying C. perfringens type A is a more common cause of food poisoning than previously known. Further studies are needed to determine whether the epidemiology of C. perfringens type A food poisonings caused by plasmid-borne and chromosomal cpe-carrying strains differs.

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