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Clostridium perfringens Type A Strains Carrying a Plasmid-Borne Enterotoxin Gene (Genotype IS*1151-cpe* or IS*1470*-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 IS*1470-cpe*), 21% (plasmid-borne IS*1470*-like-*cpe*), and 4% (plasmid-borne IS*1151-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.* IS*1470* is found downstream of the chromosomal *cpe* (1), whereas either IS*1470*-like or IS*1151* 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 Hyytiä 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 (IS*1151-cpe*, IS*1470*-like-*cpe*, or IS*1470-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 thiourea 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 IS*1470-cpe* was detected in 8 (73%) of the Finnish and 10 (77%) of the German outbreaks. The plasmid-borne IS*1470*-like-*cpe* was detected in three (27%) of the Finnish and in five (21%) of the German outbreaks, whereas the plasmid-borne IS*1511-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 IS*1470-cpe*, 21% for the plasmid-borne IS*1470*-like-*cpe*, and 4% for the plasmid-borne IS*1511-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 IS*1470*-like-*cpe* were considerably more common than the strains carrying plasmid-borne IS*1151-cpe*. Further studies are warranted to elucidate whether the phenomenon holds true globally and which factors contribute to these

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TABLE 1. Characterization of *Clostridium perfringens* type A strains isolated from food poisoning outbreaks

Outbreak	Country	Isolate[a]	Origin	PFGE pattern[b]	Presence of:		*cpe* location and genotype[e]		
					cpe[c]	CPE[d]	Chromosome IS*1470-cpe*	Plasmid IS*1151-cpe*	Plasmid IS*1470*-like-*cpe*
1	Germany	721/84	Rabbit meat	1	+	+	−	+	−
		731/84	Feces	1	+	+	−	+	−
		732/84	Feces	1	+	+	−	+	−
2	Germany	954/84	Feces	2	+	+	+	−	−
		955/84	Feces	3	+	+	+	−	−
3	Germany	20/85	Feces	4	+	ND	+	−	−
		22/85	Feces	4	+	ND	+	−	−
4	Germany	310/85	Rabbit meat	5	+	+	+	−	−
		313/85	Feces	5	+	+	+	−	−
		314/85	Feces	5	+	+	+	−	−
5	Germany	945/85	Heart goulash	6	+	+	+	−	−
		948/85	Cauliflower salad	7	+	+	+	−	−
		949/85	Cauliflower salad	7a	+	+	+	−	−
		955/85	Feces	6a	+	+	+	−	−
6	Germany	26/86	Feces	8	+	+	+	−	−
7	Germany	318/86	Feces	10	+	ND	+	−	−
		320/86	Feces	10	+	ND	+	−	−
8	Germany	497/86	Feces	11	+	ND	+	−	−
		501/86	Feces	11a	+	ND	+	−	−
9	Germany	1292/86	Meatballs	12	+	ND	+	−	−
		1293/86	Meatballs	12a	+	ND	+	−	−
10	Germany	1533/86	Feces	13	+	+	−	−	+
		1534/86	Feces	13	−	ND	−	−	−
11	Germany	834/87	Pea mash	14	+	+	+	−	−
		836/87	Feces	14a	+	+	+	−	−
12	Germany	344/91	Poultry fricassee	17	−	−	−	−	−
		346/91	Feces	NT	+	ND	+	−	−
13	Germany	147/92	Feces	18a	+	+	−	−	+
		149/92	Feces	18	+	+	−	−	+
		148/92	Feces	18	−	ND	−	−	−
		155/92	Feces	18	−	−	−	−	−
14	Finland	D9030/97	Feces	19	+	+	+	−	−
		D9031/97	Feces	19	+	ND	+	−	−
		D9032/97	Feces	19	+	ND	+	−	−
		D9033/97	Feces	20	−	ND	−	−	−
15	Finland	C215/99	Roast beef	21	+	+	−	−	+
		C216/99	Roast beef	22	+	+	−	−	+
16	Finland	C269/00	Salmon	23	+	+	−	−	+
17	Finland	C545/02	Unknown food	24	+	+	+	−	−
18	Finland	C645/03	Meatball soup	25	+	+	+	−	−
		C646/03	Meatball soup	25	+	+	+	−	−
		C647/03	Meatball soup	25	+	ND	+	−	−
		C648/03	Meatball soup	25	+	+	+	−	−
19	Finland	C746/04	Pea soup	26	+	ND	+	−	−
		C747/04	Pea soup	26	+	+	+	−	−
		C748/04	Pea soup	26	+	+	+	−	−
		C749/04	Pea soup	26	+	+	+	−	−
20	Finland	C774/04	Pork fillet	27	+	+	−	−	+
		C775/04	Pork fillet	27	+	+	−	−	+
21	Finland	C776/05	Pea soup	28	+	+	+	−	−
22	Finland	C793/05	Pork casserole	29	+	+	+	−	−
23	Finland	C796/07	Cabbage gratin	30	+	+	+	−	−
24	Finland	C797/07	Beef stroganoff	31	+	+	+	−	−

[a] The identification number of the isolate and the year of isolation (1984 [84] to 2007 [07]).
[b] PGFE patterns of the isolates. NT, not typeable.
[c] +, positive; −, negative by PCR in determining the presence of *cpe* gene.
[d] +, positive by PET-RPLA; ND, not determined due to unsuccessful sporulation.
[e] +, positive by genotyping PCR; −, negative by genotyping PCR.

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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