CLOSTRIDIUM PERFRINGENS

THE ORGANISM/TOXIN

Clostridium perfringens are anaerobic rod-shaped, Gram-positive bacteria associated with foods such as rolled meat joints, stews and gravies.

During cooking, vegetative cells of *C. perfringens* die but they form spores able to survive the unfavourable conditions. If the food is cooled slowly, the spores can germinate into vegetative cells that can then multiply to high numbers. When the highly contaminated food is eaten, although the cells are unable to grow in the gut they form spores and as the cells break to release the spore, a large amount of toxin is released into the gut (Czeczulin *et al.,* 1993; McClane, 2007). Symptoms include watery diarrhoea and abdominal pain that usually resolves itself as the diarrhoea removes the toxin from the body.

There are five types of *C. perfringens* based on toxin type (A, B, C, D, E). Most *C. perfringens* food poisoning cases reported in developed countries are caused by type A strains. Type C causes a rare, severe, necrotic enteritis (Bates and Bodnaruk, 2003).

Less than 5% of all *C. perfringens* carry the gene responsible for the production of the enterotoxin causing Type A illness (the *cpe* gene). Only strains carrying this gene in a chromosome (C-*cpe*) cause foodborne illness (Sarker *et al*., 2000; Wen and McClane 2004; McClane, 2007). This datasheet covers only Type A and *cpe* positive *C. perfringens*.

GROWTH AND CONTROL

Isolates with chromosomal *cpe* have a competitive advantage over isolates with the same gene located on a plasmid (P-*cpe*), as they are more resistant to several food preservation procedures (e.g. heat, refrigeration, freezing) (Li and McClane, 2006b; Novak and Yuan, 2003).

Optimal heat shock occurs around 75°C which will activate germination of any spores in the food. Slow cooling and/or slow reheating provide germinating spores and any surviving vegetative cells with conditions (>10°C to <54°C) allowing multiplication to large numbers, particularly as competitive flora will have been killed (McClane, 2007).

Growth

Temperature

- Minimum 10°C
- Maximum 54°C
- Optimum 43°C (Li and McClane, 2006b)

Some isolates have a very short generation time (<10 min.) (Bates and Bodnaruk, 2003; McClane, 2007; Andersson *et al.,* 1995).

Enterotoxin is produced only during spore formation but remains intracellular until lysis of the mother cell to release the mature spore. Toxin is not produced in significant amounts during optimum vegetative growth. Toxin production is optimum at 35-40^{o}C.

pH

- Optimum 6-7
- Range 5.1 to 9.7

Spore formation occurs between pH 5.1 and 9.9 (Li and McClane 2006a).

Atmosphere

- Optimal under anaerobic conditions.

Small amounts of oxygen, up to a redox potential of +200 mV, can be tolerated, but generation and lag times lengthen (Juneja *et al.,* 1994). The redox potential of many foods, including meat, is sufficient to allow growth to start, after which the atmosphere is made more anaerobic by the organism. Growth rates are similar under carbon dioxide or nitrogen atmospheres (Parekh and Solberg, 1970).

Minimum Water Activity

- 0.93 (Labbe, 1989)

Survival

Temperature

Concentrations of C-*cpe* vegetative cells decline at refrigeration temperatures, with C-*cpe* isolates surviving longer; $D_{4°C}$ of 11 days compared to 1.8 days for P-*cpe* isolates (Li and McClane, 2006b). Similarly, the $D_{-20°C}$ was 1.5 days for C-*cpe* cells compared to 0.6 days for P-*cpe* isolates.

Spores survive both refrigeration (4°C) and freezing (-20°C) with less than 1-log reduction in spore viability after 6 months at both refrigeration and freezing temperatures.

pH

Sporulation occurs between pH 6 and 8 under gut conditions. The hardy spores show less than a 1.2 log decrease in numbers after 3 months at pH 4 and pH 10.

Atmosphere

C. perfringens vegetative cells survive some exposure to oxygen (redox potentials between +200 to +300 mV).

Inactivation

Temperature

A literature review indicated a mean $D_{70°C}$ of 23 seconds with a 95th percentile of 125 seconds (based on 146 datapoints) (van Asselt and Zwietering, 2006). Cooking for 2 minutes at 70°C would achieve a mean (approximate) 6-log reduction in vegetative cells, but would not kill spores. In the same review, a mean $D_{120°C}$ of 18 seconds was calculated for spores, with a 95th percentile of 161 seconds (based on 64 datapoints).

D-values for different isolates of *C. perfringens* vary, particularly when present as spores, and C-*cpe* strains appear more hardy. As spores, the $D_{100°C}$ of six C-*cpe* strains was between 30 and 124 min compared with 0.5-1.9 min for P-*cpe* strains (Sarker *et al*., 2000).

While the enterotoxin is a heat-labile protein and inactivated by heating for 5 min at 60^{o}C, it is not generally produced in food (McDonel, 1986).

pH

- Vegetative C-*cpe* cells inactivated below pH 5 (Bates and Bodnaruk, 2003)
- C-*cpe* spores slow inactivation below pH 5
- Enterotoxin not generally produced in food

Preservatives

For the curing of meats, hurdle technology is often used. For example, the use of pH (6.2), salt (1%) and sodium nitrite (50µg/ml) act synergistically to inhibit *C. perfringens* vegetative growth at 15°C (Gibson and Roberts, 1986).

Disinfectants / Sanitisers

- Most food industry sanitisers destroy vegetative *C. perfringens* cells on surfaces.
- Glutaraldehyde, formaldehyde, chlorine, iodine, acids, alkalis, hydrogen peroxide, peroxy acids, ethylene oxide, ß-propionolactone and ozone are all sporicidal at high concentrations with long contact times.
- Phenolics, QACs, alcohols, bisguanides, organic acids, esters and mercurials have little sporicidal effect.

Chlorine disinfectants such as household bleach contain 5.25% sodium hypochlorite (52,500 ppm available chlorine) and are effective against vegetative *C. perfringens* but not against spores.

CLINICAL PICTURE

Incubation: 6-24 hours, usual onset 10-12 hours.

Symptoms: Profuse watery diarrhoea with severe abdominal pain that subsides within 24 hours or less. Diarrhoea is initiated by the enterotoxins causing tissue-damage to intestinal cells. Vomiting and nausea are rare. Severity of illness is lessened by the diarrhoea flushing out both the enterotoxin and sporulating cells from the small intestine (McClane, 2007). Estimated hospitalisation and fatality rates are 0.3% and 0.05%, respectively.

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Condition: Gastroenteritis or *C. perfringens* enteritis.
Dose: Approximately10^8 organisms. Large numbers of vegetative cells must be ingested. Assuming a 100g serving, at least 10^6/g of food is needed to cause illness (ICMSF, 1996; McClane, 2007).
Toxins: Illness is due to foodborne infection with toxin produced in the intestine. Rare cases of intoxication with pre-formed toxin have been reported, resulting in earlier onset of symptoms. In instances where the bacteria have multiplied to such levels that sporulation and toxin production occurs, the food is usually in an advanced state of spoilage (Bates and Bodnaruk, 2003; McClane, 2007). Up to 15% of a sporulating cell's protein can be enterotoxin.
At Risk Groups: No specific 'at risk' groups.
Long Term Effects: Generally none.
Treatment: Not usually given as symptoms resolve naturally (McClane, 2007).

SOURCES

Human: USA, Finnish and Japanese studies indicate that most healthy adults are not routinely colonised by *C. perfringens* C-*cpe* type strains but about half of all subjects studied were colonised with non-enterotoxigenic type A vegetative cells and/or spores, including P-*cpe* strains (Carman *et al.,* 2008). In general, counts in populations of healthy individuals of $<10^3$-10^4/g faeces are considered normal (Bates and Bodnaruk, 2003). The organism is more numerous in healthy neonates and the elderly than in adults. Food handlers are not thought to be a source of food contamination as the organism already exists on the at-risk foods. As levels of *C. perfringens* spores can be elevated in some healthy individuals (e.g. the elderly), detection of toxin in faeces is preferable to culture for diagnosis and outbreak investigation. Analysis for enterotoxin should be carried out within 48 hours of onset due to rapid elimination of toxin from the gut. PCR methods on suspected food samples are also useful because they identify the presence of the C-*cpe* gene (Bates and Bodnaruk, 2003).
Animal: *C. perfringens* can be found in the contents of virtually all animal intestines. Contamination of carcasses occurs at slaughter. Animal foods are the most common vehicles.
Food: *C. perfringens* can be found in raw, dehydrated and cooked foods. Type A food poisoning often involves large volumes of food, especially meat and poultry dishes, prepared and cooked (possibly undercooked) in advance then allowed to cool too slowly. Cooking creates an anaerobic atmosphere as oxygen is depleted. Rolled meats, stuffed poultry, pies, thick soups, stews, gravies and curries have been implicated in outbreaks. In the case of rolled meats, any bacteria on the outside of the meat are rolled into the centre where conditions are anaerobic and heat can be slow to penetrate. Undercooking of foods is a major factor for survival of vegetative cells. Heating activates any spores while slow cooling promotes germination and growth.
The most effective way to prevent *C. perfringens* food poisoning is to prevent its growth in food, i.e. strict controls on cooling and on storage temperatures.
Environment: Spores of *C. perfringens* are resilient and are widely distributed in soil, dust and vegetation.
Transmission routes: Primarily ingestion of food with high levels of *C. perfringens* vegetative cells ($>10^6$/g).

OUTBREAKS AND INCIDENTS

No particular seasonal patterns. Outbreak investigations commonly reveal control point failures including preparation too far in advance (implying temperature abuse), undercooking, inadequate cooling, poor reheating and improper hot holding.

NZ Incidence: While cases of food poisoning due to *C. perfringens* are not notifiable, outbreak statistics are recorded. In 2009, 3 outbreaks were attributed to the organism (88 cases) accounting for 3/586 enteric outbreaks and 3/84 foodborne outbreaks in New Zealand (ESR, 2010).

<u>New Zealand Outbreaks</u>
Butter chicken and rice: 78 cases. Pre-prepared meals delivered to workplaces. Inadequate cooling or refrigeration.
Chicken biryani and mutton curry: 58 cases. Social function. Inadequate cooling and reheating, improper hot holding.
Seafood mornay: 121 cases. College. Inadequate cooling and reheating, improper hot holding.
Roast meat and gravy: 4 cases. Restaurant. Inadequate cooling of beef stock, poor hot holding practices.
Roast turkey: 57 cases. Restaurant. Inadequate cooking, cooling and holding procedures, insufficient reheating.

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