Clostridium perfringens

Clostridium perfringens
Family of *Clostridiaceae*

Bacterium

anses
French agency for food, environmental and occupational health & safety

Characteristics and sources of *Clostridium perfringens*

Main microbial characteristics

Large, box-shaped rods (1 to 15 µm in diameter), non-motile, spore-forming, Gram positive, with strict but aerotolerant anaerobic metabolism. *C. perfringens* rarely sporulates in usual culture media, only in special sporulation media, but sporulates fairly easily in a natural environment (the intestine, or soil).

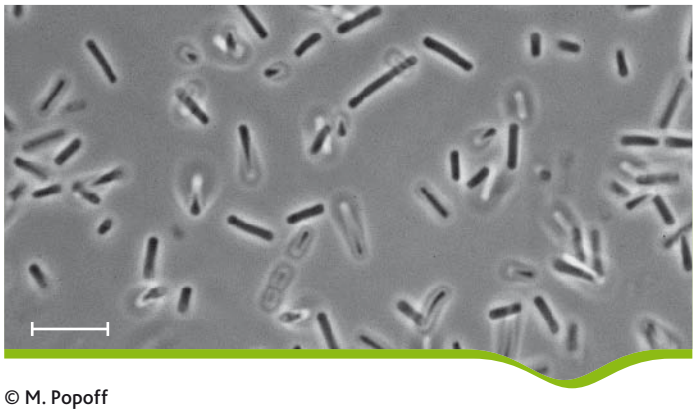
Cultures emit a considerable amount of gas, and sulfites are reduced (black colonies in the presence of sodium sulfite and iron alum). *C. perfringens* is glycolytic (acidification of glucose, lactose and maltose, in particular) and proteolytic.

C. perfringens produces and secretes numerous toxins and hydrolytic enzymes including the enterotoxin responsible for food poisoning which, unlike the other toxins of *C. perfringens*, is only synthesised during sporulation. The strains of *C. perfringens* are usually classified into 5 toxinotypes (A, B, C, D and E) according to the main toxins produced, but genotyping shows greater diversity between strains. Approximately 6-8% of strains of all origins possess the enterotoxin gene.

C. perfringens develops easily in complex peptone media but much less in defined media. Under optimal conditions it can double in 7 min.

Table 1. **Characteristics concerning survival, growth and toxin production**

Parameters	Growth		Enterotoxin and spores
	Optimum	Extremes	Stability
Temperature (°C)	40-45	10-52	Spores: conditions for sporulation not clearly known and variable, depending on strains. Enterotoxin: thermolabile (destroyed in a saline solution by heating for 5 min at 60°C)
pH	6-7	5-8.3	
a_w		Lower limit: 0.95/0.97	
NaCl (%)	3	2-6.5% Inhibiting concentration 6-8%	



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Sources of the hazard

C. perfringens is a highly ubiquitous bacterium widely distributed in all types of environment (soil, sediments, sewage, slurry, carcasses, dust, the surfaces of plants, etc.).

Healthy humans and animals can be carriers of *C. perfringens* in their digestive tubes. But the quantity of *C. perfringens* in their digestive contents is low, 10 to 10^3/g.

C. perfringens is a frequent contaminant of food products, especially those of animal origin. These can be contaminated either during the evisceration phase at the slaughterhouse, or from a contaminated environment (work surface, contact with contaminated foods, dust, etc.).

Furthermore, *C. perfringens* causes many severe illnesses in animals, especially necrotic enteritis in young piglets, poultry and, more rarely, the young of other species, enterotoxaemia in sheep, cattle and sometimes other species, dysentery in lambs.

Transmission routes

Humans become infected by transmission of the bacteria from cooked dishes, especially meat dishes. Food poisoning by *C. perfringens* occurs only after the consumption of foods heavily contaminated by an enterotoxigenic strain of this bacterium (see "Role of foods" section). No cases of direct transmission between sick animals and humans have been reported, nor between sick and healthy humans.

C. perfringens can also contaminate wounds and cause gangrene.

Data sheet on foodborne biological hazards
December 2010

Human foodborne illness

Nature de la maladie

The characteristics of the disease are presented in Table 2.

Only the enterotoxigenic strains of *C. perfringens* are responsible for food poisoning. If a large quantity of *C. perfringens* is ingested, the bacteria can become implanted in the small intestine. Some of the bacteria ingested are destroyed in the stomach (very acid pH, environment rich in protease) and the digestive flora resident in the intestine blocks their development. However, if too many are ingested, a fraction of *C. perfringens* can survive passage through the stomach and multiply in the contents of the small intestine, reaching 10^8 to 10^9 bacteria/g. *C. perfringens* then sporulates there and synthesises the enterotoxin which, liberated after lysis from the bacterial wall, interacts with the enterocytes, causing water and electrolytes to leak out, and necrosis. As a result, *C. perfringens* is found in large numbers (higher than 10^6/g) in the faeces of patients. The enterotoxin is also present in faeces during the symptomatic phase of the illness.

Susceptible population groups(1): studies with healthy volunteers have shown that all individuals are susceptible to poisoning following ingestion of food contaminated by *C. perfringens*. No immunity appeared after repeated exposure.

C. perfringens is also a causative agent of severe gangrene and puerperal septicaemia in humans. Alpha toxin and perfringolysin are the main toxins implicated in gangrenes.

Dose-response relationship(2)

The foods or culinary preparations responsible for food poisoning contain at least 10^5 live vegetative forms of enterotoxigenic *C. perfringens* per gram, the concentration at which there is the possibility of multiplication in the host's small intestine, sporulation and production of enterotoxin (see above). The expression of the enterotoxin gene is co-regulated together with that of the sporulation genes. Contaminated food does not contain preformed enterotoxin, as *C. perfringens* does not usually sporulate in culinary preparations.

Epidemiology

In France, *C. perfringens* has the fourth largest number of outbreaks (2006-2007), and the highest number of cases in 2006 and the third highest in 2007 (Table 3) among the causes identified in the framework of obligatory notification of foodborne illness outbreaks (source InVS(3)). It should be emphasised that many outbreaks are undoubtedly not notified or not diagnosed. The notification system for foodborne illness outbreaks is the only source of statistics in France about food poisoning by *C. perfringens*, and concerns only outbreaks. Most notified foodborne illness outbreaks occur in institutional catering (in 2006-2008, more than 80% of outbreaks caused by *Bacillus cereus* or *Clostridium perfringens* occurred in collective catering). The main foods incriminated in *C. perfringens* foodborne illness outbreaks are cooked dishes (37% of all outbreaks in which *C. perfringens* was incriminated), meat dishes (23%) and poultry (12%). In 20% of these outbreaks, it was not possible to incriminate a particular food.

No French or foreign sources compile sporadic cases.

In North America and the Scandinavian countries, *C. perfringens* is the second or third most common cause of foodborne illness outbreaks.

Table 3. InVS data (2008) concerning foodborne illness outbreaks caused by *C. perfringens* in France

	2006		2007	
	Confirmed	Suspected	Confirmed	Suspected
Outbreaks	11 (4%)	31 (8.9%)	33 (11.2%)	36 (9.1%)
Cases	389 (13.4%)	815 (22.9%)	789 (22.6%)	605 (14.3%)
Hospitalised	2 (0.1%)	6 (0.2%)	10 (2.8%)	4 (1.9%)

% of total foodborne illness cases.

Role of foods

Main foods to consider

Foods involved

These are most often foods prepared in advance and in large quantities. The most typical example is meat in gravy, cooked in large volumes and in advance, which has not been cooled rapidly enough between preparation and serving. Preparations with a high starch content, such as beans, and especially beans served in sauce, also represent a risk.

Conditions leading to contamination

Raw materials are usually only slightly contaminated, well below the threshold presenting a risk of poisoning (10^5/g). Cooking conditions and subsequent storage of prepared food are determinant factors in the change in the level of contamination.

Although cooking destroys most vegetative forms, it destroys no, or few, spores. One effect of boiling is to favour the removal of gases from the food being prepared, thus providing sufficiently anaerobic conditions for the growth of *C. perfringens*. Large volume preparations are especially propitious in producing this result, as re-oxygenation through contact with ambient air is slower than with small volumes.

Since *C. perfringens* multiplies rapidly in a medium rich in meat or starch within a temperature range of 30 to 50°C, keeping prepared food within this temperature range for several hours enables this bacteria to proliferate beyond the critical threshold of 10^5/g.

(1) Susceptible population groups: people with a higher than average probability of developing symptoms or severe forms of the disease, after exposure to a foodborne hazard [definition used for ANSES datasheets].

(2) Relationship between the dose (the quantity of microbial cells ingested during a meal) and the effect on an individual.

(3) French Institute for Public Health Surveillance.

Table 2. Characteristics of the disease

Mean incubation period	Target populations	Main symptoms	Duration of symptoms
6-24h (generally 10-12h)	All consumers of the foods implicated (see above, points "Epidemiology" and "Main foods to consider" irrespective of age and gender)	Diarrhoea (90-100%) Violent stomach pains (80-100%) Nausea (occasional) Vomiting (rare) Fever (rare)	1-3 days
Duration of infectious period	**Complications**	**Asymptomatic forms**	
The phase during which *C. perfringens* is carried in the digestive tube can be long, but this is not a contagious phase, as there is no direct transmission to healthy subjects.	Mortality observed in elderly people and infants (rare)	Possibility of healthy *C. perfringens* carriers	

Inactivation treatments in industrial environments

Table 4. **Inactivation of *C. perfringens***

Disinfectants	Effects of temperature
Sodium hypochlorite - concentration recommended to destroy spores: 1%	D-value* for the spores of *C. perfringens* (varies with strains): • $D_{100\,°C}$ = 0.2 – 43 min • $D_{95\,°C}$ = 1.3 – 63 min
High Pressure	**Irradiation**
No data	No data

* D is the time needed to divide by 10 the initial population of a microbial hazard.

Contamination of raw materials such as meat usually does not exceed 10 to 10^2 CFU/g. It does not seem possible to obtain produce that is systematically free of *C. perfringens*. Preventive measures must therefore be based on controlling its proliferation in cooked dishes.

The principal measure is to control the time spent by ready-made meals in the temperature range +10°C to +63°C (see box "Recommendations to operators").

In collective catering, prepared food must be cooled rapidly so that the core temperature does not remain between +63°C and +10°C for more than two hours, unless a validated hazard analysis has proved that less rapid cooling is sufficient to guarantee the safety of products of animal origin and any foods containing them. After cooling, these products of animal origin and foods containing them must be stored in a closed container at a temperature between 0°C and +3°C.

Prepared foods to be served hot should be reheated in such a way that their temperature does not remain between +10°C and the serving temperature for more than one hour.

Monitoring in foods

The NF EN ISO 7937 standard (2005) gives instructions for counting *C. perfringens* in food, in tryptose-sulfite-cycloserine agar in anaerobiosis conditions at 37°C, with confirmation of colonies at 46°C in lactose-sulfite broth.

Routine microbial surveillance of *C. perfringens* is of limited utility in the prevention of risks of foodborne illness.

Recommendations to operators

- **Observe good hygiene practice.**
- **In collective catering facilities:**
  - **cool prepared food rapidly to ensure that the core temperature does not remain between +63°C and +10°C for more than two hours;**
  - **reheat prepared food to be served hot rapidly to ensure that the temperature does not remain between +10°C and the serving temperature for more than one hour. Under no circumstances should the latter temperature be lower than +63°C, unless a validated hazard analysis has proved that a lower temperature entails no risk to the health of consumers. Prepared dishes must be consumed on the day on which they are first reheated.**

Domestic hygiene

- Respect the cold chain for foods.
- Ready-made meals must be cooled as rapidly as possible and consumed within a short period (one day) or be refrigerated (4°C), but only for a relatively short period (2-3 days).

Recommendations to consumers

- **The greatest risk concerns preparations involving meat dishes with gravy.**
- **General hygiene for preparing foods.**
  - **if meals are prepared in advance, cool rapidly (refrigerate within two hours, maximum) after cooking and keep in a refrigerator (4°C) or freezer. If a large quantity of food has been prepared, it should be separated into smaller portions so that cooling occurs more rapidly;**
  - **reheat dishes prepared in advance rapidly.**

References and links

General references

- Brunestad S, Granum P. E. *Clostridium perfringens* and food borne infections. Int. J. Food Microbiol. 2002, 74:195-202.
- Labbe R. *Clostridium perfringens*. In Foodborne Bacterial Pathogens, Doyle M. P. (Ed.), Marcel Dekker, New York, 1989, pp 191-234.
- EFSA J. *Clostridium* spp in foodstuffs 2005, 199: 1-65.
- Morbidité et mortalité dues aux maladies infectieuses d'origine alimentaire en France [Morbidity and mortality due to infectious foodborne illnesses in France], InVS report, March 2004.
- Les toxi-infections alimentaires collectives en France entre 2006 et 2008 [Foodborne illness outbreaks in France between 2006 and 2008]. BEH no. 31-32. 27 July 2010.

Useful links

- National Reference Centre for Anaerobic Bacteria and Botulism, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15.
  http://www.pasteur.fr/sante/clre/cadrecnr/anaer-index.html
- http://www.invs.sante.fr/surveillance/tiac
- http://www.anses.fr