Clostridium perfringens

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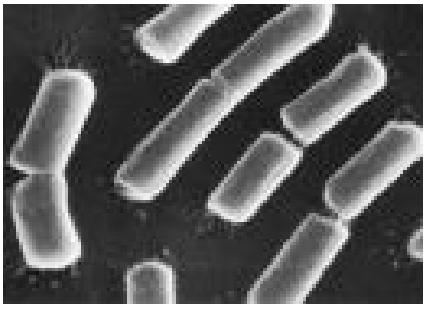
(materials from M. N. Hajmeer)

Clostridium perfringens



Outline

- Historical background
- *C. perfringens* characteristics
- Foodborne disease estimates
- Classification
- Pathogen prevalences
- Clinical features & mechanism of disease
- *C. perfringens* enterotoxins and spores
- Immunity, reservoirs, shedding, growth/survival, and detection

Background

- 1890s, F.W. Andrewes and E. Klein were involved in linking *Clostridium welchii* (now *C. perfringens*) with food poisoning.
- They associated eating foods contaminated with *C. perfringens* with several foodborne outbreaks.
- Outbreaks were characterized with mild to severe diarrhea and abdominal pain.

Background

- 1892: The microorganism was found in a variety of diseases.
  - gas gangrene
  - appendicitis
  - puerperal fever (infection of the placental site following delivery or abortion)
  - enteritis

Background

- 1939 to 1946: Several outbreaks observed:
  - In the U.K. during WWII
  - Shortage of meat led to the practice of cooking meat for later consumption.

Background

- 1939 to 1946: Several outbreaks observed:
  - First warning of food poisoning came from Knox and Macdonald (1943).
  - Vehicle: gravy made the previous day was heavily contaminated with anaerobic sporing bacilli including *C. perfringens*.
  - Children became ill after eating contaminated meal.

Background

- 1939 to 1946: Several outbreaks observed:
  - In the US, the first proven outbreak of *C. perfringens* was described by McClung (1945).
  - Examined 4 foodborne outbreaks associated with eating chicken steamed 24 hrs before consumption.

Background

- 1948: Severe necrotic gastroenteritis occurred in Germany.
  - The vehicle for the illness was home canned rabbit.
  - The associated strain was *C. perfringens* type C.

Background

- 1953: Hobbs and associates showed that eating food contaminated with *C. perfringens* could lead to diarrheal food poisoning.
- Pioneered work establishing *C. perfringens* as a cause of food poisoning.

Background

- 1954: Experiments were conducted in US with human volunteers (Dack).
  - The strain fed was English strain of *C. perfringens*.
  - Volunteers failed to develop any symptoms of disease.
- Public health significance was not fully accepted in US until the 1960s.

Background

- By the 1960s and 70s, enough information had accumulated to indicate that *C. perfringens* foodborne poisoning is caused by the release of the toxin during sporulation of the microorganism in the intestine of infected individuals who ate food heavily contaminated with *C. perfringens*.

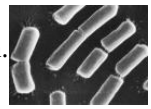
Best estimates of annual cases and deaths for foodborne diseases, USA

	Cases	Per-cent	Deaths	Per-cent
C. perfringens	**248,520**	**1.8**	7	**0.4**
Total bacterial	**4,175,565**	**30.2**	**1,297**	**71.7**
Total foodborne	**13,814,924**	**100.0**	**1,809**	**100.0**

CDC, 1998–2002: 130 outbreaks, 6,724 cases, 4 deaths.

Characteristics of *C. perfringens*

- Gram positive, nonmotile, encapsulated rods with square ends.
- Anaerobe but more oxygen-tolerant than *C. botulinum.*
- Produces acetone, butanol, ethanol, butyric acid, acetic acid, propionic acid, lactic acid, carbon dioxide and hydrogen.
- Ferments sugars, starch and pectin.



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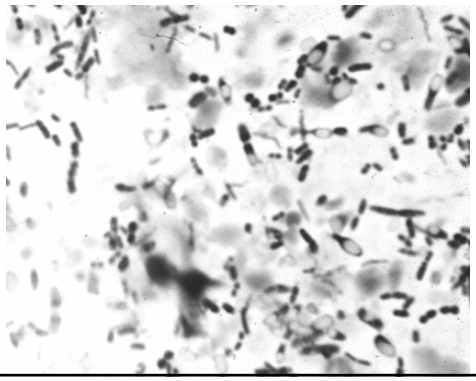

Spores and Sporulation

- Because it is a spore-former, the pathogen can survive in the environment.
- Spores are seldom formed in food.
- Sporulation requires a well-buffered medium rich in nutrients.
- Spores are formed in the intestinal tract (spores shed in feces).

Classification of *C. perfringens* is Based on Toxins Produced

Type	Toxin			
	alpha	beta	epsilon	iota
A	+	0	0	0
B	+	+	+	0
C	+	+	0	0
D	+	0	+	0
E	+	0	0	+

Classification of *C. perfringens* is Based on Toxins Produced

- The alpha toxin is a phospholipase, the others are hemolysins or cause necrosis.
- The different types cause a variety of diseases in animals, some of them very severe.

Classification of *C. perfringens* is Based on Toxins Produced

- Type A is hemolytic (α and β) and non-hemolytic.
- Killing 90% of spores takes 6 – 17 minutes at 100°C for non-hemolytic strains and less than one minute for beta-hemolytic strains.

C. perfringens Prevalence in Foods

Pork	0–39%
Cooked Pork	45%
Beef	22%
Chicken	0–54%
Seafood	2%

Clinical Features of *C. perfringens*

- Severe diarrhea; no pyrexia (fever), shivering, headache, abdominal pain, dehydration
- Incubation time to illness is 8-24 h.
- Duration of illness is 12-24 h.

Clinical Features of *C. perfringens*

- Large numbers of the pathogen can be found in feces and food.
- Significant amount of enterotoxin also found in feces.
- Infective dose is high. About 10^8 vegetative cells need to be ingested to cause symptoms.

Mechanism of Disease

- *C. perfringens* produces toxin-mediated infection known as *C. perfringens* enteritis.
- *C. perfringens* produces food poisoning without colonization.

Mechanism of Disease

1. *C. perfringens* in food.
2. Ingested cells begin to sporulate after passing stomach.
3. Enterotoxin produced in small intestine (during sporulation)
4. Symptoms

Mechanism of Disease

- Sporulation is poor in most foods; therefore contain little or no enterotoxin.
- Sporulation is rapid in small intestines where pH is right and well buffered. It begins in the intestines in 3 h, and enterotoxin found in 10-12 h.

Mechanism of Disease

- Heat shock, 70 – 100 °C, boosts germination and yields of enterotoxin.
- If food such as meat containing spores is heated and left for some time at growth temperature the "primed" spores will produce rapidly growing vegetative cells that in turn will produce plenty of spores and enterotoxin in the small intestines.

Mechanism of Disease

- Avoid problems by boiling food immediately before eating to:
  - ➢Kill the vegetative cells in the food, and
  - ➢Prevent sporulation in the intestines

Mechanism of Disease

- *C. perfringens* food poisoning outbreaks tend to be on a large scale
  - ➢ large volumes of food produced
- There are few if any fatalities.

C. perfringens Enterotoxins

- Simple polypeptide, molecular weight of 36000 ± 4000, contains 309 amino acid residues but only one cysteine residue
- They are inactivated by pronase and *B. subtilis* protease but not by trypsin, chymotrypsin, papain or bromelin.
- Enterotoxin is heat labile with 90% destruction in 4 minutes at 60°C.

C. perfringens Enterotoxins

- Have similarities with *V. cholerae* enterotoxin
  - ➢Cause a transient increase in capillary permeability with fluid accumulation and diarrhea
  - ➢Increased secretion of water, sodium and chloride
  - ➢Decreased absorption of glucose
  - ➢Enterotoxin causes desquamation of villous epithelium.

C. perfringens Enterotoxins

- About 0.2 – 36 μg toxin/g feces is found in patients suffering from *C. perfringens* food poisoning.
- Only toxigenic types A and C (seldom D) of *C. perfringens* produce enterotoxin (causes gastrointestinal symptoms).

C. perfringens Spores

- *C. perfringens* spores can be isolated from healthy persons.
- These spores are probably formed in the colon rather than in the small intestines.

Immunity

- After an incident of disease, circulating antibodies that neutralize enterotoxin are found in the blood but not in the intestines.
- *C. perfringens* food poisoning does not confer immunity.

Reservoirs

- *C. perfringens* is found in human and animal intestinal tracts, and soil.
- ~ 50% to 100% of normal, healthy humans are carriers of *C. perfringens.*
- Carriers excrete around 10^3 spores/g of feces.
- Recovering individuals shed $>10^5$/g.

Shedding Frequency

- Shedding frequency in animals:
  - Swine 18%
  - Rats 41%
  - Chicken 88% (60% produced toxin)
  - Cattle 80% (68 % produced toxin)

Growth and Survival

- Growth temperature is 6.5 – 47°C
- Freezing cells at –18°C: only 4% survived for 180 days.
- Freezing spores at –18°C: only 11% survived for 180 days.
- Storage at 5°C more lethal than freezing at –18°C.

Survival and Growth

- A 1.2–3.4 kGy of gamma rays kills 90% of spores.
- Water activity of 0.95–0.96 limits growth.
- The limiting NaCl concentration is 5–8%
- Upper redox potential permitting growth +31 mV at pH 7.7, +230 mV at pH 6

Detection of *C. perfringens*

- Many different media have been proposed
  - see Compendium of Methods for the Microbiological Examination of Foods
- Sulfite cycloserine agar is convenient for pour plates.
- Neomycin blood agar for surface plating.

Detection of *C. perfringens*

- Most probable numbers (MPN) can be determined in cooked meat or liver medium followed by streaking on neomycin blood agar.
- Enterotoxin can be detected by ELISA test.