



## Introduction

- ◆ At least three gram-positive sporeforming rods are known to cause bacterial food poisoning: *Clostridium perfringens* (產氣莢膜梭菌), *C. botulinum* (肉毒桿菌), and *Bacillus cereus* (仙人掌桿菌).



## *CLOSTRIDIUM PERFRINGENS* *FOOD POISONING*

- ◆ Gram-positive, anaerobic spore-forming rod
- ◆ Widely distributed in nature.
- ◆ *C. perfringens* food poisoning → **large numbers of viable cells must be ingested** → **Food infection**
- ◆ Based on their ability to produce certain **exotoxins**, five types are recognized: types **A, B, C, D, and E**.



## *CLOSTRIDIUM PERFRINGENS* *FOOD POISONING*

- ◆ The **food-poisoning strains belong to type A**. They are generally **heat resistant** and produce **only traces of alpha toxin** (Not to be confused with aflatoxin. 黃麴毒素).
- ◆ *Clostridium perfringens* alpha toxin, a membrane-disrupting toxin, which is directly responsible for gas gangrene (氣性壞疽) and myonecrosis (肌肉壞死).
- ◆ Some type C strains produce **enterotoxin** and may cause a food-poisoning syndrome.



## Distribution of *C. perfringens*

- ◆ Exist in **soils, water, foods, dust, spices**, and the **intestinal tract** of humans and other animals.
- ◆ The **heat-resistant, nonhemolytic strains** to range from 2% to 6% in the general population.
- ◆ The **heat-sensitive** types are common to the **intestinal tract of all humans**.



## Distribution of *C. perfringens*

- ◆ *C. perfringens* gets into **meats** directly from **slaughter animals** or by **subsequent contamination** of slaughtered meat from containers, handlers, or dust.
- ◆ **A spore former → withstand drying, heating, and certain toxic compounds.**



## Characteristics of the Organism

- ◆ **Facultative anaerobic**
- ◆ Mesophilic with an **optimum between 37°C and 45°C**. The lowest temperature for growth is around **20°C**, and the highest is around **50°C**.
- ◆ Many strains grow over the **range 5.5-8.0** but generally not below 5.0 or above 8.5.
- ◆ The **lowest reported water activity ( $a_w$ )** values for growth and germination of spores lie **between 0.97 and 0.95** with sucrose or NaCl, or **about 0.93 with glycerol**.



## The Enterotoxin

- ◆ The *C. perfringens* enterotoxin (CPE) is a **spore-associated protein** → produced during sporulation in the **GI tract (gastrointestinal tract)**.
- ◆ Food poisoning due to **type A strains** has been **fatal only in elderly or otherwise debilitated (虛弱的) persons**.



## The Enterotoxin

- ◆ The purified enterotoxin is **heat sensitive** (biological activity **destroyed at 60°C for 10 min**) and pronase (a protease from *Streptomyces griseus*) sensitive but resistant to trypsin (胰蛋白酶), chymotrypsin (胰凝乳蛋白酶), and papain (木瓜蛋白酶).



## Mode of Action

- ◆ The *C. perfringens* enterotoxin (CPE) is not a superantigen as are the staphylococcal enterotoxins
- ◆ Enterotoxigenesis begins when **CPE binds to protein receptors on epithelial cells** in the gastrointestinal tract. → **associates with a membrane protein** to form a larger complex → **CPE-induced membrane permeability alterations** → lead to **death of host cells**.



## Vehicle Foods and Symptoms

- ◆ Symptoms appear between 6 and 24 hours, especially between 8 and 12 hours, after the ingestion of contaminated foods.
- ◆ The symptoms are characterized by **acute abdominal pain (急性腹痛) and diarrhea**; nausea, fever, and vomiting are rare.



## Vehicle Foods and Symptoms

- ◆ Except in the elderly or in debilitated persons, the illness is of **short duration—a day or less**. The **fatality rate is quite low**, and **no immunity seems to occur**, although circulating antibodies to the enterotoxin may be found in some persons with a history of the syndrome.



## Vehicle Foods and Symptoms

- ◆ The foods involved in *C. perfringens* outbreaks are often **meat dishes prepared one day and eaten the next**. The heat preparation of such foods is presumably **inadequate to destroy the heat-resistant endospores**, and when the food is cooled and rewarmed, the **endospores germinate and grow**.



## Prevention

- ◆ - Same as in chapter 23
- ◆ kept either *below 40°F (4.4°C)* or *above 140°F (60°C)* until consumed



## BOTULISM

- ◆ Botulism is caused by certain strains of *C. botulinum*, a **gram-positive, anaerobic spore-forming rod**.
- ◆ On the basis of the serological specificity of their toxins, seven types are recognized: A, B, C, D, E, F, and G. Types A, B, E, F, and G cause disease in humans.



## BOTULISM

- ◆ Unlike *C. perfringens* food poisoning, in which **large numbers of viable cells must be ingested (→ Food infection)**, the symptoms of **botulism** are mostly caused by the **ingestion of a highly toxic, soluble exotoxin** produced by the organism while growing in foods. → **Food intoxication**





## Distribution of *C. botulinum*

- ◆ Soils and waters.
- ◆ All **toxin-producing strains** have been placed into one of four groups—I, II, III, or IV.
  - Group I contains the proteolytics (types A and G; some B and F )
  - Group II the nonproteolytics (types E; some B and F)
  - Group III consists of types C and D.
  - Group IV serological type G.



## Growth of *C. botulinum* Strains

- ◆ The proteolytic strains → digest casein and produce H<sub>2</sub>S.
- ◆ The nonproteolytic strains → ferment mannose
- ◆ The nutritional requirements → amino acids, B vitamins, and minerals
- ◆ The proteolytics **generally do not grow below 12.5°C. The upper range is about 50°C.**



## Growth of *C. botulinum* Strains

- ◆ The nonproteolytic strains can grow as low as 3.3°C.
- ◆ Growth does not occur at or below pH 4.5
- ◆ The minimum  $a_w$  that permits growth and toxin production → 0.94 ~ 0.97.
- ◆ Proteolytic strains are much more heat resistant than nonproteolytics.



## Ecology of *C. botulinum* Growth

- ◆ This organism cannot grow and produce its toxins in competition with large numbers of other microorganisms.
- ◆ In the presence of yeasts, however, *C. botulinum* has been reported to grow and produce toxin at a pH as low as 4.0. ← Yeasts are presumed to produce growth factors needed by the clostridia to grow at low pH.



## Nature of the Botulinal Neurotoxins

- ◆ The **neurotoxins are formed within the organism and released upon autolysis** (the self-digestion that occurs in tissues or cells by enzymes in the cells themselves).
- ◆ They are produced by cells growing under **optimal conditions**, although resting cells have been reported to form toxin as well.



## Nature of the Botulinal Neurotoxins

- ◆ **The botulinal neurotoxins (BoNT) are the most toxic substances known.**
  - The minimum lethal dose for mice has been reported also to be 0.4-2.5 ng/kg by intravenous (靜脈內的) or intraperitoneal (腹腔內的) injection, and a 50% human lethal dose of about **1 ng/kg** of body weight has been reported.



## Nature of the Botulinal Neurotoxins

- ◆ The genes for BoNT A, B, E, and F are **chromosomal**, whereas type G is **plasmidborne**.
- ◆ Symptoms of botulism can be produced by either parenteral (非腸道的) or oral administration of the toxins. They may be **absorbed into the blood stream** through the **respiratory mucous membranes**, as well as through **the walls of the stomach and intestines**.



## Nature of the Botulinal Neurotoxins

- ◆ Unlike the staphylococcal enterotoxins and heat-stable toxins of other foodborne pathogens, the botulinal toxins are **heat sensitive** and may be **destroyed by heating at 80°C (176°F) for 10 minutes**, or **boiling temperatures for a few minutes**.



## The Adult Botulism Syndrome: Incidence and Vehicle Foods

- ◆ Symptoms of botulism may develop anywhere **between 12 and 72 hours** after the ingestion of **toxin-containing foods**.
- ◆ **Symptoms** consist of nausea, vomiting, fatigue (疲勞), dizziness (頭昏眼花), and headache; dryness of skin, mouth, and throat; constipation (便秘), lack of fever, paralysis (麻痺) of muscles, double vision, and, finally, **respiratory failure and death**.



## The Adult Botulism Syndrome: Incidence and Vehicle Foods

- ◆ Treatment consists of administering **specific antisera** as early as possible.
- ◆ **Heat suspect foods to boiling temperatures for a few minutes**, which is sufficient to destroy the neurotoxins.



## Infant Botulism

- ◆ In the adult form of botulism, preformed toxins are ingested.
- ◆ In infant botulism, **viable botulinal spores are ingested**, and upon germination in the intestinal tract, toxin is then synthesized.



## Infant Botulism

- ◆ Although it is possible that in some adults under special conditions botulinal endospores may germinate and produce small quantities of toxin, **the colonized intestinal tract does not favor spore germination.**
- ◆ Infants over **1 year of age** tend not to be affected by this syndrome because of the establishment of a more normal intestinal biota.



## ***BACILLUS CEREUS*** **GASTROENTERITIS**

- ◆ *Bacillus cereus* is a gram-positive, **aerobic**, **spore-forming rod**
- ◆ Present in soil, dust, and water.
- ◆ Low numbers of this bacterial species can be found in a number of food products, including fresh and processed.



## ***BACILLUS CEREUS*** **GASTROENTERITIS**

- This bacterium has a minimum growth temperature around **4 - 5°C**, with a maximum around **48-50°C**. Growth has been demonstrated over the **pH range 4.9-9.3**. Its spores possess a resistance to heat.



## *B. cereus* Toxins (Food intoxication)

- ◆ This bacterium produces a wide variety of **extracellular toxins** and enzymes, including lecithinase (卵磷脂酶), proteases,  $\beta$ -lactamase (乙内酰胺酶), sphingomyelinase (鞘磷脂酶), cereolysin (mouse lethal toxin, hemolysin I), and hemolysin BL.



## *B. cereus* Toxins (Food intoxication)

- ◆ The **diarrheagenic syndrome** appears to be produced by a tripartite complex composed of components B, L1, and L2 and designated **hemolysin BL (HBL)**. → exhibits hemolysis (溶血), cytolysis (細胞溶解), dermonecrosis (皮膚壞死), vascular permeability (血管滲透性), and enterotoxic activity. **The HBL is attributed to heat-labile (不耐熱的) enterotoxins.**





## *B. cereus* Toxins (Food intoxication)

- ◆ The **emetic toxin** strains grow over the range **15-50°C**, with an optimum between **35°C and 40°C**.
- ◆ The emetic toxin is an **ionophoric**, water-insoluble peptide. Its activity is lost after heating **30 minutes at 121°C**.
  - An **ionophore** (離子載體) is a lipid-soluble molecule that transports ions across a cell membrane.
  - The emetic syndrome is most often associated with **rice dishes**.



## Diarrheal Syndrome

- ◆ This syndrome is rather mild, with symptoms **developing within 8-16 hours**, more commonly within 12-13 hours, and lasting for 6-12 hours.
- ◆ **Symptoms** consist of **nausea (with vomiting being rare)**, **cramp-like abdominal pains** (腹絞痛), tenesmus (感覺急需大小便而無法順利排出之現象), and **watery stools**. **Fever is generally absent**.
- ◆ The similarity between this syndrome and that of ***C. perfringens* food poisoning** has been noted.
  - Symptoms appear between 6 and 24 hours, especially between 8 and 12 hours, after the ingestion of contaminated foods. The symptoms are characterized by **acute abdominal pain** (急性腹痛) and **diarrhea**; nausea, fever, and vomiting are rare



## Diarrheal Syndrome

- Vehicle foods consist of **cereal dishes** that contain corn and corn starch, mashed potatoes, vegetables, minced meat, liver sausage, meat loaf (美式肉餅), milk, cooked meat, Indonesian rice dishes, puddings, soups, and others.



## Emetic Syndrome

- ◆ **Emetic syndrome** of *B. cereus* food poisoning is **more severe and acute than the diarrheal syndrome**. The incubation period ranges from **1 to 6 hours**, with 2 to 5 hours being most common.
- ◆ Its similarity to the **staphylococcal food-poisoning syndrome** has been noted. It is often associated with **fried or boiled rice dishes**.



- ◆ 香港首例！醫美打肉毒桿菌 女銀行家喪命 (2018/11/13)
- ◆ 4人肉毒桿菌中毒 婆媳吃豆乾後發病 (2010/5/22)
  - 真空包裝豆乾、素肚
  - 工廠與販售點未冷藏食品、製造日期亂標
  - 建議同學們食用前先加熱煮沸數分鐘
- ◆ 胡蘿蔔沒洗淨，5月嬰肉毒桿菌中毒 (2015-11-28)
  - 冷凍胡蘿蔔與地瓜塊帶有表皮與泥沙，推測泥土中的菌體與芽孢混入食品。
  - 用胡蘿蔔、地瓜製作嬰兒副食品，最好新鮮現做洗乾淨，反覆解凍易致細菌感染。