

# Spore-Forming Gram-Positive Bacilli: Bacillus and Clostridium Species

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# Bacillus Species

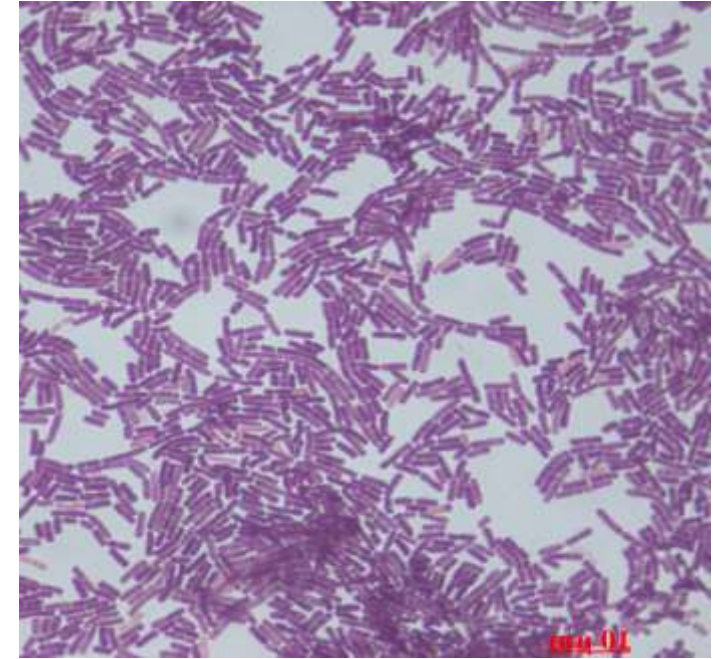
- ❖ The genus *Bacillus* includes large aerobic or facultatively anaerobic, gram-positive, spore forming rods occurring in chains.
- ❖ Saprophytic, prevalent in soil, water, and air, such as *Bacillus cereus* and *Bacillus subtilis*.
- ❖ Some are insect pathogens, such as *B. thuringiensis*.
- ❖ *B. anthracis*, which causes anthrax, *B. cereus* are the principal pathogens of the genus.

# *Bacillus cereus*

- Gram-positive aerobic or facultatively anaerobic, motile, spore-forming, rod-shaped bacterium that is widely distributed environmentally.
- *B. cereus* is associated mainly with food poisoning.
- *B. cereus* has also been associated with localized and systemic infections, including endocarditis, meningitis (Transplant patients), osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections.
- Enterotoxins are usually produced by bacteria outside the host and therefore cause symptoms soon after ingestion of *B. cereus*.

# Morphology and identification

- A 3–4  $\mu$  m, arranged in long chains; spores are located in the center of the motile bacilli.
- *B. cereus* can be differentiated from *B. anthracis* on the basis of colony morphology,  $\beta$ -hemolysis, motility, produce licithenase and antimicrobial susceptibility patterns.



*B. anthracis*

# Epidemiology

- The heat-resistant spores of *B. cereus* are widespread and contaminate **rice** and other cereals. the spores germinate if left at room temperature.
- A heat-stable toxin can also be produced which can survive “flash frying”.
- The natural environmental reservoir for *B. cereus* consists of decaying organic matter, fresh and marine waters, vegetables and fomites, and the intestinal tract of invertebrates, from which soil and food products may become contaminated, leading to the transient colonization of the human intestine .
- Spores germinate when they come into contact with organic matter or within an insect or animal host.

# Pathogenesis

- Secreted toxins : hemolysins , distinct phospholipases, an emesis-inducing toxin, and three pore-forming enterotoxins: hemolysin BL (HBL), nonhemolytic enterotoxin (NHE), and cytotoxin K.

# Food poisoning

- ❖ Food poisoning caused by *B cereus* has two distinct forms :
  - The emetic type, which is associated with fried **rice** & cereals.
  - The diarrheal type, which is associated with meat dishes and sauces.
- ❖ The enterotoxin may be preformed in the food or produced in the intestine.

# Clinical features

- There are two clinical syndromes produced by the toxins:

1- vomiting type –heat stable toxin(cerulide): Incubation period 0.5–6 hours ,occasionally diarrhea and cramps can occur. The illness is usually self-limiting and over in 24 hours.

2 - The diarrheal type-Heat labile toxin: Incubation period 6–15 hours followed by an illness similar to that seen with *C. perfringens*. The diarrhea and abdominal cramps may be associated with nausea (vomiting is rare) but are over in 24 hours.



# Diagnosis and treatment

- Clinical grounds.
- Isolation of *B. cereus* from the suspect food, as well as from the stool or vomitus of the patient.
- Culture and Gram stain of implicated material.



# Treatment and prevention

- Food-poisoning is self-limiting, therefore antimicrobial therapy is not normally required.
- *B cereus* is resistant to a variety of antimicrobial agents, including penicillins and cephalosporins.

# CLOSTRIDIUM SPECIES

- Spores of clostridia are usually wider than the diameter of the rods in which they are formed. Most species of clostridia are motile and possess peritrichous flagella.
- Clostridia are anaerobes; a few species are aerotolerant. In general, the clostridia grow well on the blood-enriched media or other media used to grow anaerobes.

# Species of Medical Importance

- *Clostridium tetani* -tetanus, Rigid paralysis.
- *Clostridium botulinum*-botulism, flaccid paralysis.
- *Clostridium perfringens*-gas gangrene & Food poisoning.
- *Clostridium difficile* -pseudomembranous colitis.

# Clostridium botulinum

## ❖ Distinguishing Features:

- Anaerobic Endospore-forming gram-positive bacilli.
- Botulism is characterized by symmetrical, descending, flaccid paralysis of motor and autonomic nerves usually beginning with cranial nerves.
- Habitat : Since it is found in the soil, it may contaminate vegetables cultivated in or on the soil. It also colonizes the gastro-intestinal tract of fishes, birds and mammals .

# Pathogenesis

## ❖ Botulinum toxin:

- Highly toxic neurotoxin-Coded for by a prophage-
- Seven Serotypes (A-G) based on the antigenicity of the botulinum toxin produced.

# Mechanism of action

- The most common offenders are spiced, smoked, vacuum packed, or canned alkaline foods that are eaten without cooking. In such foods, spores of *C botulinum* germinate; that is, under anaerobic conditions, vegetative forms grow and produce toxin.
- Absorbed by gut and carried by blood to peripheral nerve synapses.
- Blocks release of acetylcholine at the myo-neuronal junction resulting in a reversible flaccid paralysis.

# Botulism

❖ There are five clinical categories of botulism:

1) Foodborne botulism.

2) Wound botulism.

3) Infant botulism.

4) Adult infectious botulism.

5) Inadvertent, following botulinum IM toxin injection.





# Clinical Findings

- Initial symptoms can include nausea, vomiting, abdominal cramps or diarrhea that begin 18–36 hours after ingestion of the toxic food.
- Dry mouth, blurred vision, and diplopia are usually the earliest neurologic symptoms. They are followed by inability to swallow, and speech difficulty. In severe cases, extensive respiratory muscle paralysis leads to ventilatory failure.
- The infants in the first months of life develop poor feeding, weakness, and signs of paralysis (floppy baby). Infant botulism may be one of the causes of sudden infant death syndrome.

# Diagnosis

- Toxin can often be demonstrated in serum, gastric secretions, or stool from the patient, and toxin may be found in leftover food using ELISAs and PCR.
- Mouse bioassay is the test of choice for the confirmation of botulism.

# Treatment

- Supportive treatment, especially adequate mechanical ventilation, is of prime importance in the management of severe botulism .
- Surgical debridement in wound botulism.
- Antitoxin administration .A trivalent (A, B, E) anti-toxin must be promptly administered intravenously with supportive care .
- Although most infants with botulism recover with supportive care alone, antitoxin therapy is recommended.

# Prevention, and Control

- Canned food must be sufficiently heated to ensure destruction of spores .
- The risk from home-canned foods can be reduced if the food is boiled for more than 20 minutes before consumption.
- No honey for the first year infants.

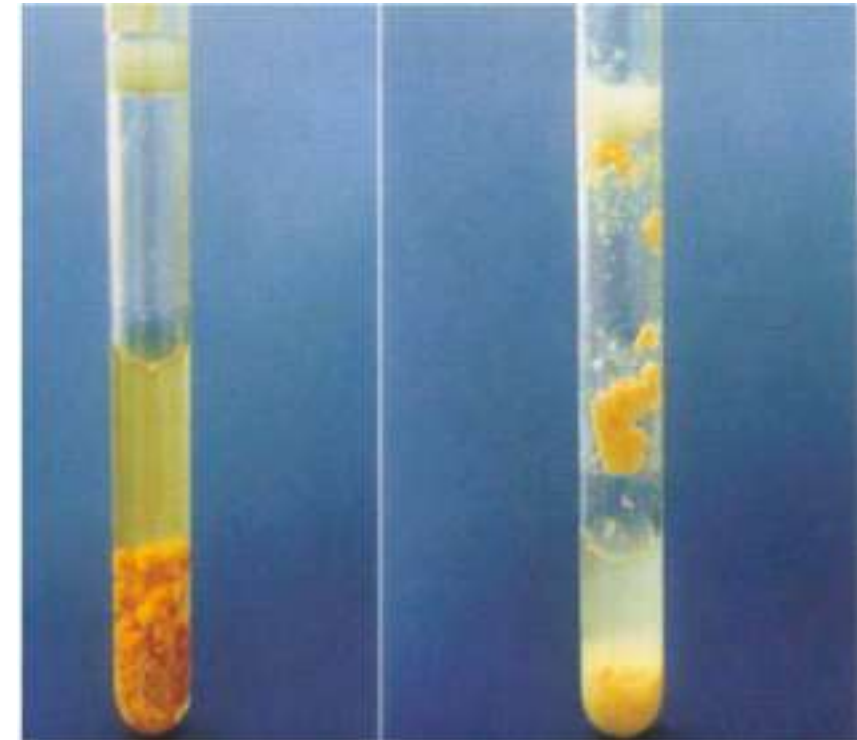
# Clostridia that produce invasive infections

- Many different toxin-producing clostridia can produce invasive infection (including myonecrosis and gas gangrene) if introduced into damaged tissue. About 30 species of clostridia may produce such an effect, but the most common in invasive disease is *C. perfringens* (90%). An enterotoxin of *C. perfringens* is a common cause of food poisoning .



# Distinguishing Features

- Large gram-positive, spore-forming rods (spores rare in tissue), non-motile
- Anaerobic: "stormy fermentation" in milk media
- Double zone of hemolysis
- Reservoir-soil and human colon
- Transmission---foodborne and traumatic implantation



# Epidemiology

- *C. perfringens* is widely present in the environment, in the intestine of humans and domestic animals and can contaminate meat during preparation for consumption. Small numbers of microorganisms may survive subsequent cooking particularly in large pieces of meat, and multiply during the cooling down and storage resulting in food poisoning.
- A more serious but rare illness (necrotizing enteritis or pigbel disease) is caused by ingesting food contaminated with Type C strains.

# Pathogenesis

- In invasive clostridial infections, spores reach tissue either by contamination of traumatized areas (soil, feces) or from the intestinal tract. The spores germinate at low oxidation reduction potential; vegetative cells multiply, ferment carbohydrates present in tissue, and produce **gas**.
- Toxins have lethal, necrotizing, and hemolytic properties. The  $\alpha$  and  $\theta$  toxins. Some strains of *C. perfringens* produce a powerful enterotoxin as well.

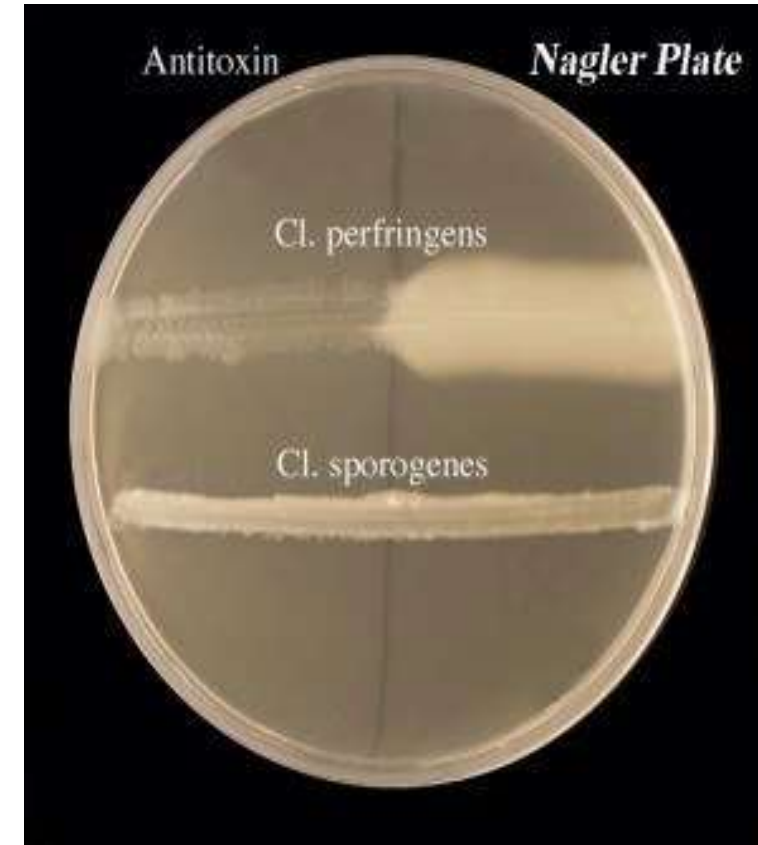


# Clinical Findings

- From a contaminated wound (eg, a compound fracture, postpartum uterus), the infection spreads in 1–3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death.
- *C perfringens* food poisoning usually follows the ingestion of large numbers of clostridia that have grown in warmed meat dishes. The toxin forms when the organisms sporulate in the gut, with the onset of diarrhea—usually without vomiting or fever—in 7–30 hours. The illness lasts only 1–2 days.

# Diagnostic Laboratory Tests

- Gram-stained smears of specimens from wounds, pus, and tissue.
- Culture material into thioglycolate medium and onto blood agar plates incubated anaerobically. The growth from one of the media is transferred into milk. *C. perfringens* rarely produces spores when cultured on agar in the laboratory.
- Final identification rests on toxin production and neutralization by specific antitoxin.e.g. Nagler test.



# Treatment and prevention

- Prompt and extensive surgical debridement of the involved area and excision of all devitalized tissue, in which the organisms are prone to grow.
- Administration of antimicrobial drugs, particularly penicillin, is begun at the same time. Hyperbaric oxygen may be of helpful. It is said to “detoxify” patients rapidly.
- Antitoxins are available against the toxins of *C perfringens*, usually in the form of concentrated immune globulins. Antitoxins should not be relied on.
- Food poisoning caused by *C perfringens* enterotoxin usually requires only symptomatic care.

# Clostridium difficile infection (CDI)

# Epidemiology

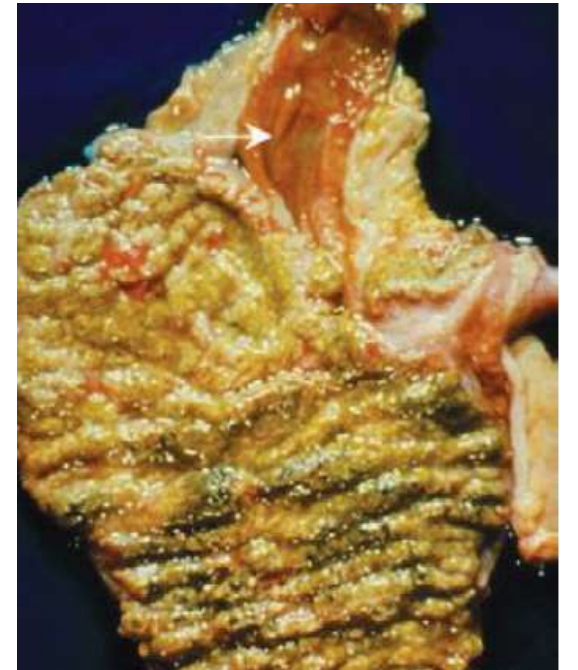
- Ubiquitous in the environment and colonizes the intestine of 50% of healthy neonates and 4% of healthy adults.
- A major cause of healthcare-associated infection; patients taking antibiotics, e.g. cephalosporins, clindamycin are at increased risk of developing *C. difficile* antibiotic associated diarrhea.
- This is due to suppression of the normal bowel flora and subsequent overgrowth of *C. difficile*. Infection may be endogenous or exogenous (through ingestion of environmental spores).

# Pathogenesis

- Produces two major toxins: Toxin A (enterotoxin) and Toxin B (cytotoxin).
- Toxin A induces cytokine production with hypersecretion of fluid.
- Toxin B induces depolymerisation of actin with loss of cytoskeleton. Adhesin factor and hyaluronidase production are also associated virulence factors.
- Hypervirulent, hypertoxin producing strains now recognised (e.g. ribotype 027, 078).

# Disease

- Antibiotic associated diarrhoea,
  - Mild to moderate.
- Pseudomembranous colitis (PMC), fulminant colitis.
  - Severe forms



# Diagnosis

- ❖ The diagnosis of CDI is based on a combination of clinical criteria:
  - (1) diarrhea ( $\geq 3$  unformed stools per 24 h for  $\geq 2$  days) with no other recognized cause plus,
    - (2) toxin A or B detected in the stool (e.g. ELISA, latex agglutination, and polymerase chain reaction (PCR )) or culture of *C. difficile* on selective agar
    - (3) pseudomembranes seen in the colon.
- ❖ PMC is a more advanced form of CDI and is visualized at endoscopy in only ~50% of patients with diarrhea who have a positive stool culture and toxin assay for *C. difficile*.



# Treatment and prevention

- Discontinue other antibiotics therapy.
- Oral administration of vancomycin or metronidazole is recommended for CDI treatment.
- Caution in overprescribing broad-spectrum antibiotics (limited-spectrum drugs should be considered first).
- In the nursing home setting, patients who are symptomatic should be isolated.
- Autoclave bed pans (treatment kills spores).

The End