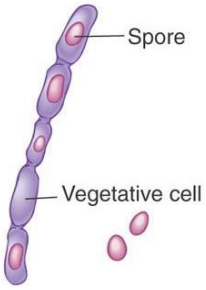
Spore-Forming Gram-Positive Bacilli

(*Bacillus* & *Clostridium*)

The family Bacillaceae consists of rod-shaped Gram-positive bacteria that form endospores. The family includes two main groups of spore-forming bacteria:

- The anaerobic spore-forming bacteria of the genus *Clostridium.*
- The aerobic or facultatively anaerobic spore-forming bacteria of the genus *Bacillus*.

1- Bacillus



The genus *Bacillus* is frequently known as aerobic spore bearers. They are ubiquitous and are present in soil, dust, air, and water. These bacteria are also frequently isolated as contaminants in bacteriological culture media. The genus *Bacillus* consists of more than 50 species. ***Bacillus anthracis*** and ***Bacillus cereus*** are the two most important species that cause infections in humans and animals. *B. anthracis* causes **anthrax**, while *B. cereus* causes **food poisoning**.

B. anthracis - Virulence factors

Two virulence factors –

- Capsular polysaccharide – inhibits phagocytosis
- Anthrax toxin: made up of 3 fractions

1. **Protective antigen factor (PA or Factor II)**
2. **Edema factor (EF or Factor I)**
3. **Lethal factor (LF or Factor III)**

* They are not toxic individually; the whole complex produces local edema & generalized shock. Toxin production is plasmid mediated. Human Anthrax may present as

1. **Cutaneous anthrax:** 'Hide Porter's disease' – inoculation of spores into the skin
2. **Pulmonary anthrax:** 'Wool Sorter's disease' – inhalation of spores

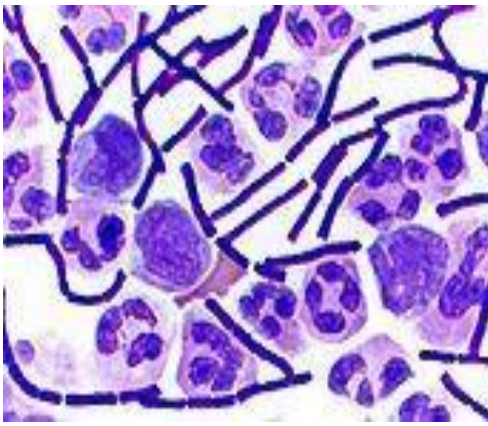
3. **Gastrointestinal anthrax** - ingestion of inadequately cooked meat containing anthrax spores

* All types lead to fatal septicemia

Laboratory Diagnosis

- **Specimens**

Vesicular fluid (fluid from under the eschar), Blood, lymph node, or splenic aspirates, CSF & Sputum.



- **Microscopy**

Gram-stained smear of **a skin lesion** show encapsulated, broad, large, Gram-positive bacilli. The bacteria are found as single, in pairs, and in short chains. The bacilli do not show any spores**. In smears from culture,** *B. anthracis* grows as long chains and may appear similar to streptobacilli. The bacilli are arranged end-to-end and the ends of the bacilli are truncated, not rounded, or often concave and somewhat swollen. This gives the chain of bacilli a "bamboo-stick" appearance.

- **Culture**

Macroscopic appearance of serpentine chains of bacterial colony (medusa head colony). Non-hemolytic & Sticky consistency of colonies on blood agar (Fig. 1). On nutrient agar after 24 hours of incubation, *B. anthracis* produces grayish and granular colonies. Knisely's Polymyxin B-lysozyme EDTA thallous acetate (PLET) agar medium is a selective medium used for isolation of *B. anthracis* from mixtures containing other spore-bearing bacilli. The medium is composed of heart infusion agar, polymyxin, lysozyme, ethylene diamine tetra acetic acid (EDTA), and thallous acetate.

Other identifying features:

1. No growth on chloral hydrate agar.
2. Giving an 'inverted fir tree' appearance in a gelatin stab (Fig. 2).
2. Non-motile and capsulated bacteria.
3. Lysis by gamma-phage positive (Fig. 3).

4. String-of-pearls test positive.

5- Catalase positive.

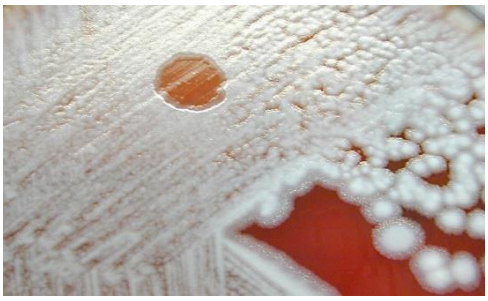


Fig. 3

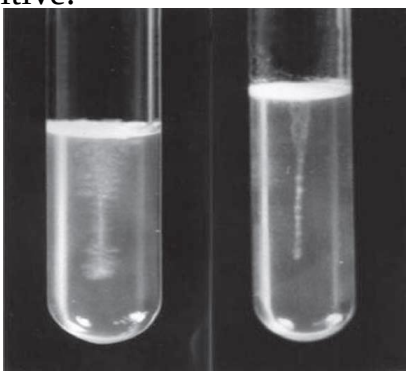


Fig. 2

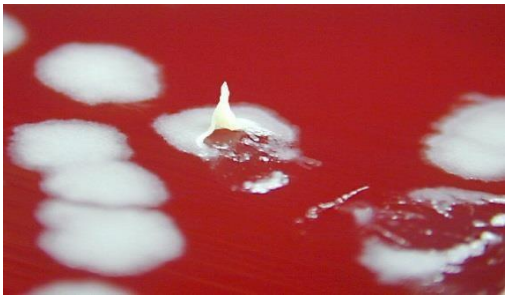


Fig. 1

Bacillus cereus

B. cereus is the most important pathogen known to cause food poisoning. *B. cereus* is a normal inhabitant of the soil, but it can be regularly isolated from foods, such as grains and spices. Episodes of *B. cereus* food poisoning occur sporadically worldwide. This occurs due to the ingestion of contaminated food in which bacteria have multiplied to high levels under conditions of improper storage after cooking. *B. cereus* causes foodborne gastroenteritis – 2 patterns of disease (diarrheal & emetic).

B. cereus is a spore-forming Gram-positive bacillus. It is generally motile, but nonmotile strains may also occur. It is a facultative anaerobe. It is a no fastidious bacterium that grows on ordinary media, such as nutrient agar. Mannitol, egg yolk, phenol red polymyxin agar (MYPA) is the selective media used for the isolation of *B. cereus* from feces and other sources. *B. cereus* ferments glucose, but not mannitol, and produces the enzyme lecithinase. *B. cereus* spores can survive in the soil over a long period.

***B. cereus* - Virulence factors**

- **The heat-labile enterotoxin:** The toxin stimulates adenyl cyclase-cAMP system causing profuse watery diarrhea.
- **The heat-stable enterotoxin:** causes emetic form of *B. cereus* food poisoning
- **Other toxins**

(a) cerolysin,a potent haemolysin, (b) necrotic toxin; a heat-labile toxin, (c) phospholipase C; a potent lecithinase.

Identifying features of *Bacillus cereus*

- *B. cereus* is a spore-forming Gram-positive bacillus.
- It is generally motile, but nonmotile strains may also occur.
- It is a facultative anaerobe.
- It is a nonfastidious bacterium that grows on ordinary media, such as nutrient agar.
- Produce β-hemolysis on blood agar

Differentiating features between *Bacillus anthracis* and *Bacillus cereus*

Characteristics	*Bacillus anthracis*	*Bacillus cereus*
Motility	Nonmotile	Motile
Capsule	Capsulated	Noncapsulated
Medusa head colony	Present	Absent
Hemolysis on sheep blood agar	Absent	Present
Growth on chloral hydrate agar	Absent	Present
Gelatin liquefaction	Slow	Rapid
String-of-pearls test	Positive	Negative
Lysis by gamma phage	Positive	Negative

Spore-Forming Gram-Positive Bacilli

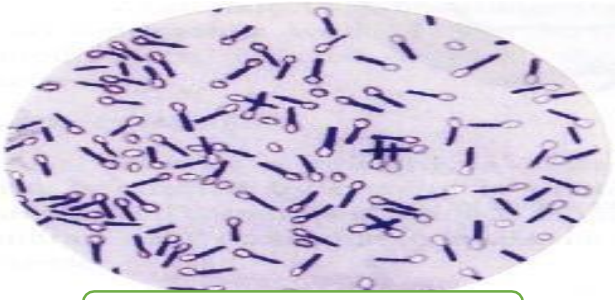
2- *Clostridium*

The genus *Clostridium* consists of Gram-positive, anaerobic bacilli capable of forming endospores. The endospores typically are wider than the bodies of the bacilli, giving the bacteria a swollen appearance resembling a spindle, hence the name *Clostridium* (*kloster*, spindle). Whilst many Clostridium species exist, only some are of medical importance, including *C. tetani, C. perfringens, C. botulinum* & *C. difficile.*

a) Clostridium tetani

C. tetani, an obligate anaerobic Gram-positive bacillus, causes tetanus. Tetanus is an infectious disorder characterized by an increased muscle tone and spasms caused by the release of a neurotoxin, *tetanospasmin*, produced by *C. tetani* when it gets inoculated into humans.

- **Morphology**
  - *C. tetani* is a slender, Gram-positive bacillus measuring 4–8 µm in length. Young cultures are usually Gram-positive but old cultures are Gram-variable and even Gram-negative.
  - It is a straight bacillus with parallel sides and rounded ends. The bacillus occurs in singles and occasionally in chains. The bacteria consist of round, **terminal**, and bulging spores giving "**drumstick" appearance** to the bacillus. The spores are rarely seen even in clinical specimens from lesions or in culture media.



drumstick appearance

- **Specimens**

  The specimens include excised bits of tissue from the necrotic depths of wounds. Swabs from the wounds are not good specimens.

- **Identifying features of *Clostridium tetani***
  1. It grows anaerobically well in Robertson's cooked meat medium with production of turbidity & some gas in the medium
  2. Extremely **fine translucent film** of growth, which tends to swarm

over the entire surface of the blood agar, with producing alpha-hemolytic colonies (on prolonged incubation become beta-hemolytic due to production of tetanolysin).

2. Motile (except type VI) by peritrichous flagella and capsulated bacteria.
3. It does not ferment any sugars. It does not produce H_2S and does not reduce nitrates.

Translucent film of growth for *C. tetani*

Note: The most useful and a simple bedside diagnostic test for tetanus is "The spatula test". (Homework)?

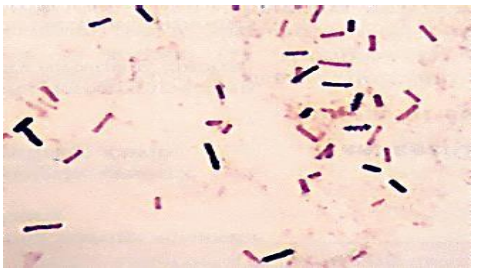
b) Clostridium perfringens

C. perfringens is the most important *Clostridium* species causing gas gangrene, a severe life-threatening disease. The bacteria also cause necrotic enteritis and food poisoning. This bacterium produces several lethal toxins and other toxins such as Enterotoxin**.** Five types (A to E) of *C. perfringens* are recognized based on surface antigens and the types of toxins produced:

1- Type A strains: commonly found in human infections; produce only α-toxin

2- Type B to E strains: commonly found in animals (lambs, goat, cattle); produce α- and other toxins.

- **Morphology**

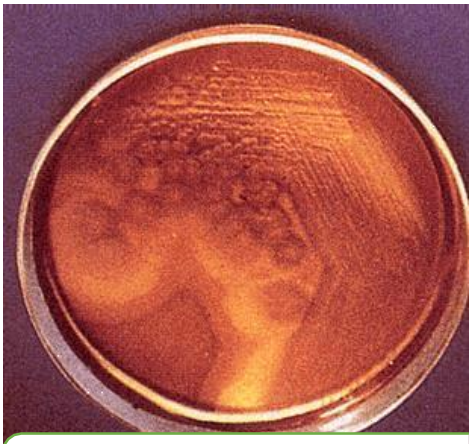


- *C. perfringens* is a large, rectangular, Gram-positive bacillus measuring 4–6 µm in length; it is a straight bacillus with parallel sides and round and truncated ends.

- They possess central or subterminal spores, The spores, however, are rarely seen either in clinical specimens from lesions or in culture media.

- **Specimens**

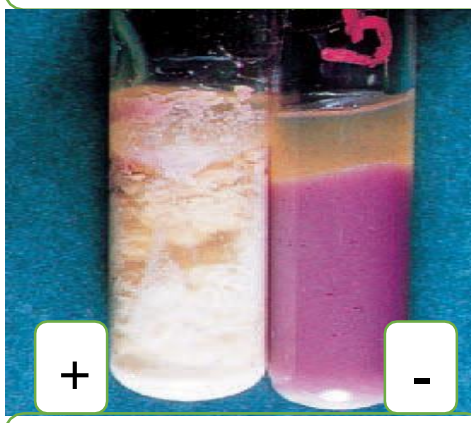
The specimens for gas gangrene include Pus and other exudates from deeper part of the wound, necrotic tissue and pieces of muscle fragments. While the specimens for investigation of food poisoning include feces and remnants of food.



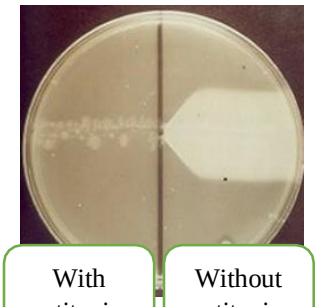
Double zone hemolysis of ***Clostridium perfringens***

➢ Identifying features of *Clostridium perfringens*

1. Grows rapidly and produces spreading colonies on solid media.
2. Produces a double zone of hemolysis around colonies on blood agar (inner zone of complete lysis due to β-toxin and wider outer zone of partial haemolysis due to α-toxin).
3. Large rectangular Gram-positive bacilli in the Gram-stained smear of the colony.
4. Nonmotile and capsulated bacteria.
5. Ferments lactose with production of acid in litmus milk medium (stormy fermentation positive).
6. They produce H_2S and reduce nitrate to nitrite.
7. Nagler reaction positive.



Stormy fermentation of ***C. perfringens*** **(left)**



With antitoxin | Without antitoxin

Nagler reaction: Nagler reaction is a useful test for rapid detection of *C. perfringens* in clinical specimens. This reaction demonstrates biological property of the enzyme lecithinase—to produce opalescence in the serum and in the egg yolk media. This reaction is specifically neutralized by the use of specific antitoxin.

c) Clostridium botulinum

C. botulinum is a heterogeneous group of spores- forming, anaerobic, Gram- positive bacteria causing botulism, a paralytic disease with the presentationof food poisoning.

➢ Morphology and identifying features

• Gram-positive bacilli with subterminal and oval bulging spores in the Gram-stained smear of the colony.
• Motile by peritrichous flagella and capsulated
• Produces large, irregular, and semitransparent colonies with irregular fimbriate border on blood agar.

- Ferments glucose, hydrolyze gelatin, and digest protein.
- Produces enzyme lipase.

➢ **Specimens**

Feces, vomitus, or gastric aspirates are collected for diagnosis of all the forms of botulism. Tissue from wound is collected for wound botulism.

➢ **Lab Diagnosis**

Microscopic detection or culture are often unsuccessful (few organisms and slow growing). Toxin detected and typed in lab via toxicity and antitoxin neutralization tests in mice or by ELISA.

d) Clostridium difficile

It is a long slender Gram-positive, anaerobic bacillus bearing large, oval, and terminal spores. It is nonhemolytic, saccharolytic, and mild proteolytic. It was named *difficile* because it grows slowly and is difficult to culture.

C. difficile is the causative agent of **antibiotic-associated diarrhea** and **pseudomembranous colitis**. *C. difficile* produces two antigenically distinct toxins: toxin A and toxin B. Toxin A is an **enterotoxin** and toxin B is a **cytotoxin**. Both the toxins contribute to pathogenesis of *C. difficile* colitis and diarrhea in humans.

Endoscopic appearance of the Pseudomembranous colitis

➢ **Lab Diagnosis**

✓ The diagnosis of *C. difficile colitis* is suspected in any patient with diarrhea who has received antibiotics within the previous 2 months and/or when diarrhea occurs 3 days or more after hospitalization.

✓ Diagnosis of *C. difficile* diarrhea is made by demonstration of *C. difficile* toxin in the feces by stool cytotoxin test and by ELISA.

✓ Stool cultures, however, are not useful due to the presence of nontoxigenic strains of *C. difficile* in feces.