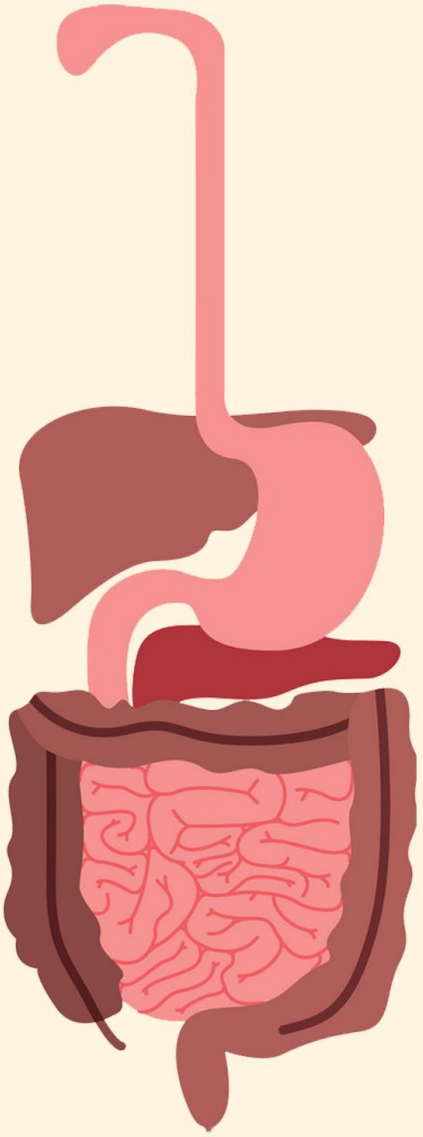
Microbiology

Doctor 2017 | Medicine | JU

Number >>	1
Doctor	Nader Alaridah
Done By	Tala Saleh
CorrectedBy	Shehab



2nd system - GI

Outline:

Two types of gram-positive, spore-forming bacilli:

1- *Bacillus "aerobic":*
   - **a-** *B. cereus*
   - **b-** *B. anthracis*

2- *Clostridium "anaerobic":*
   - **a-** *C. botulinum*
   - **b-** *C. perfringens*
   - **c-** *C. difficile*
   - **d-** *C. tetanus (not to be taken)*

Spore Forming, Gram-Positive Bacilli

- A **spore** is a structure produced during **unfavorable** condition by the process called '**sporulation'**. Under **favorable** condition spores '**germinate'** to give **vegetative** cell which grows and **produces toxins**.
- In this lecture, we will cover 2 gram-positive spore-forming rods (bacilli); **Bacillus** and **Clostridium**.
- Bacillus and Clostridium both cause disease by the release of potent exotoxins, i.e. they cause **toxin mediated diseases**.
- They differ biochemically, where **Bacillus** is **aerobic**, but **Clostridium** is generally **anaerobic**.

1- Bacillus Species

The genus Bacillus includes large **aerobic** or facultatively anaerobic, **ubiquitous** gram-positive, spore forming **rods** occurring in **chains**. Some are:

- **Bacillus cereus** and **Bacillus subtilis** → are **saprophytic** *(gets energy from decaying plants or animals)*, prevalent in soil, water, and air.
- **B. thuringiensis** → an **insect** pathogen.
- **B. anthracis and B. cereus** → are the **main** pathogens of this genus. **B anthracis** causes the disease **anthrax**, while **B cereus** causes **gastroenteritis** *(food poisoning)*.

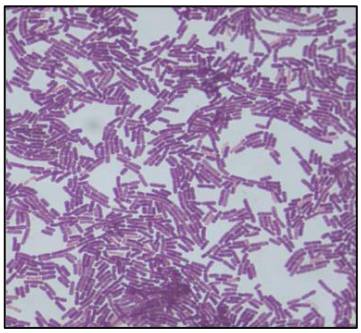
"We will only focus on B. cereus"

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, in immunocompromised patients is **fatal**, has also been associated with localized and systemic infections, such as: **endocarditis**, **meningitis** *(in 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 *(pre-formed toxins*) and therefore cause symptoms **soon** after ingestion of B. cereus.

Morphology of B. cereus:

- A 3–4 μ m, arranged in **long chains**; spores are in the **center** of the motile bacilli.
- B. cereus can be differentiated from B. anthracis based on **colony** morphology, **motility** and antimicrobial **susceptibility** patterns:



B. Cereus	B. Anthracis:
Motile and **non-encapsulated**	**Non-motile** with a **glutamic** acid capsule
Resistant to penicillin and cephalosporins	Generally, not resistant to penicillin.
Causes **food poisoning**; nausea, vomiting, and diarrhea.	Causes **anthrax**; a fatal disease of 3 forms: Cutaneous, pulmonary or gastroenteritis.
Colonies are **β-hemolytic** producing **Lecithinase**, large and more mucoid	Colonies are **non-hemolytic**, flat with a **sticky** consistency “medusa-head”.

Epidemiology:

- B. cereus forms **heat-resistant spores** that contaminate rice mainly or cereals.
- B. cereus can be found from the **environment** or **food** “rice and cereals”.
- The natural environmental reservoir for B. cereus consists of decaying organic matter “**saprophytic”**, 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 if ingested.
- Spores germinate when they encounter organic matter, within an **insect** or **animal host**.

- Food poisoning occurs when Bacillus cereus deposits its **spores** in **food** *(rice or cereal),* which survives hard conditions *(cooking)*. After that, at **room temperature**, bacteria germinate in the food and begin releasing their heat-stable enterotoxin.
- A heat-labile toxin can also be produced which can survive flash frying; fast frying with oil.

B. Cereus Pathogenesis:

B. cereus secretes:

a- **Hemolysins**; β-hemolytic.

b- Distinct **phospholipases**; Lecithinase**.**

c- **One** type of **emesis**-inducing**, heat-stable** toxin called **cereulide,** it causes **vomiting**.

d- **Three** types of **pore-forming** heat-labile **enterotoxins**, they cause **diarrhea**:

1- *Hemolysin BL (**HBL**),*

2- *Nonhemolytic enterotoxin (**NHE**)*

3- ***Cytotoxin K**.*

> ***Note:*** *Those 3 **enterotoxins** work in **synergism** (1+1> 2) causing **diarrhea**.*

B. Cereus Clinical Features:

There are two clinical syndromes produced by the previously mentioned toxins:

1- **Vomiting type** *(emetic form)* – caused by a **heat stable** and acid resistant toxin; **Cereulide**:

- Incubation period **0.5–6** hours; a **small** incubation period because the toxins are **pre-formed** when ingested, i.e. here the patient ingests the **toxin**.
- Mainly **nausea** and **vomiting** are present but **rarely** diarrhea and cramps may occur.
- The illness is usually **self-limiting** and is over within a day.
- Foods involved are: **rice** and **cereals**.

2- **The diarrheal type –** caused by the **3** heat-labile **enterotoxins**; **HBL**, **NHE**, **cytotoxin K**:

- Incubation period **6–15** hours; a **longer** incubation period because toxins are released inside the gut after ingestion, they are **not** pre-formed, i.e. here the patient ingests the **bacteria.**
- Mainly **diarrhea** and abdominal **cramps** but may be associated with nausea. Vomiting, however, is **rare**. Symptoms are over within a day.
- Foods involved are: contaminated **meat**, **vegetables** and **sauces**.

B. Cereus Diagnosis:

- Clinical grounds; the history of **food intake** would help in the diagnosis.
- Isolation of B. cereus from the **suspected food**, as well as from the vomitus of the **patient**.
- Isolation of the bacteria from **stools** is **not** diagnostic since it may be considered as a **contaminant** only, thus culturing of the suspected **food** is required to **confirm** the diagnose.

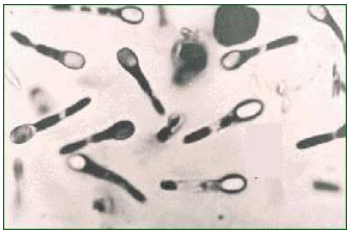
Treatment and prevention of B. Cereus:

- Food-poisoning is **self-limiting**, therefore antimicrobial therapy is **not** normally required. Fluid and electrolytes replacement are enough.
- B. cereus is **resistant** to a variety of antimicrobial agents, including **penicillin** and **cephalosporins**.
- To inactivate the spores, the cooked food must be **exposed** to **high** temperatures and/or **refrigeration**. Inactivating the spores for patients in **hospitals** is very important to prevent the **spread** of the disease.

---------- *End of Bacillus species* ----------

2- Clostridium Species

- Clostridium are also gram-positive endospore-forming rods. However, they are **anaerobic** unlike aerobic Bacillus species. They are **ubiquitous** and is part of the **microbiome** *(C. difficile).*
- A few Clostridium species are **aerotolerant**; meaning that they do **not** need oxygen but **can survive** in its presence.
- In general, the clostridia grow well on the **blood-enriched media** or other media used to grow anaerobes.
- **Spores** of clostridia are usually **wider** than the **diameter** of the **rods** and generally **subterminal** in position. Recall how in Bacillus species spores were **central**.



- Most species of clostridia are **motile** and possess **flagella**.
- Clostridium harm their human hosts by secreting **extremely powerful exotoxins** and enzymes.

Clostridium Species of Medical Importance:

1- C. Tetani: Disease → Tetanus Characterized by → Rigid paralysis.

2- C. Botulinum: Disease → Botulism Characterized by → Flaccid paralysis.

3- C. Perfringens: Disease → Gas gangrene and rarely gastroenteritis.

4- C. Difficile: Disease → Pseudomembranous colitis *(severe form)*, diarrhea *(mild form).*

1- Clostridium Botulinum

- It is found in the **soil**, which may contaminate vegetables cultivated in or on the soil. It also **colonizes** the gastrointestinal tract of fishes, birds and mammals.
- It causes **Botulism**, which is characterized by **symmetrical**, **descending**, **flaccid** paralysis of motor and autonomic nerves, usually beginning with cranial nerves.

C. Botulinum Pathogenesis:

- Clostridium botulinum produces an **extremely lethal** neurotoxin *(coded by a prophage)* even in low quantities, it causes a rapidly **fatal** food poisoning.
- There are **seven** serotypes (A-G) based on the **antigenicity** of the botulinum toxin produced. A, B, E and rarely F are the serotypes that causes the disease in humans.

C. Botulinum Mechanism of action:

- The most common offenders are **spiced**, **smoked**, **vacuum packed**, or **canned** alkaline foods that are eaten **without proper** cooking. In such foods, spores of C. botulinum germinate when placed under **anaerobic** conditions *(jar, can, etc.)* where it then matures and produce toxin.
- It gets absorbed by gut and carried by blood to peripheral nerve synapses.
- The neurotoxins block the release of **Ach** from presynaptic nerve terminals in the autonomic nervous system and motor endplates, causing a **reversible flaccid** muscle paralysis.

Botulism:

There are four clinical categories of botulism:

1- **Foodborne botulism** *(most common)*; ingesting contaminated food with **pre-formed toxins**.
2- **Wound botulism**; traumatic, most commonly amongst **drug abusers**.
3- **Infant botulism**; most common offender is **honey** followed by powdered milk. Bacteria here germinates and produce the toxins **inside the gut** rather than being pre-formed as in foodborne botulism.
4- **Unintentional**, following botulinum **IM toxin injection**; Botox injections.

Clinical Findings of Botulism:

- **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** *(double vision)* are usually the **earliest neurologic** symptoms. They are **followed** by the inability to swallow, and speech difficulty.
- **In severe cases**, extensive respiratory muscle paralysis leads to ventilatory failure and eventually death.
- **In infants,** in the first months of life, poor feeding, weakness, and signs of paralysis ***(floppy baby)*** develop.
- Infant botulism may be one of the causes of **sudden** infant **death** syndrome.

- There are **no** prominent **gastrointesinal** symptoms and no **fever** in Botulism.

C. Botulinum Diagnosis:

- Clinical grounds; exploring food intake history.
- **Bacteria** can often be demonstrated in the **serum**, gastric **secretions**, or **stool** from the patient. Toxin may be found in **leftover food** using **ELISAs** and **PCR**.
- **Mouse bioassay** is the **test of choice** for the confirmation of botulism in vitro. The mouse will be injected with the patient's serum, if it dies then this confirms botulism.

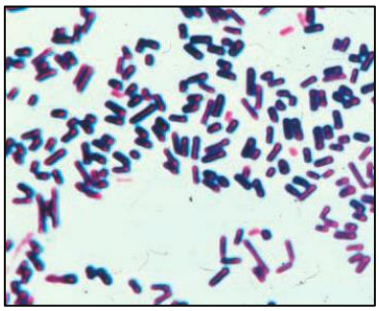
Treatment of C. Botulinum:

- **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) antitoxin must be promptly administered intravenously with supportive care.
- Although most infants with botulism recover with supportive care **alone**, antitoxin therapy is **recommended**.

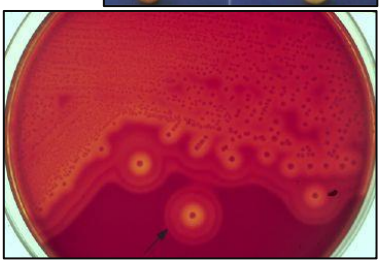
C. Botulinum Prevention:

- **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.

2- Clostridium Perfringens



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



C. Perfringens Distinguishing Features:

- Large gram-positive, spore-forming, capsulated and **non-motile** rods.
- **Anaerobic**: **Stormy fermentation** in milk media where coagulation and bubbles appear.
- **Double zone** of hemolysis.
- Reservoir-soil and human colon.
- Transmission: Foodborne and traumatic implantation.
- Spores are **rarely** seen in culture media.

C. Perfringens 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 **anaerobic conditions,** vegetative cells then multiply, ferment carbohydrates present in tissue, and **produce gas**.
- **Deep wounds** create an **anaerobic** environment that offers an excellent home for C. perfringens. As this anaerobic organism grows, it releases **exotoxins** inside the tissue causing further destruction.
- Toxins have **lethal**, **necrotizing**, and **hemolytic** properties. C. perfringens produces **alpha**, **epsilon** and **theta** toxins. These toxins are associated with **soft tissue infections**.
- Some strains of C. perfringens are associated with **intestinal diseases.** They sporulate **inside** the **gut** under nutritionally deprived conditions producing a powerful **sporulation-associated enterotoxin.**

> ***Note:*** *There are **2 types** of soft tissue **infections** caused by C. perfringens. One is **wound infections**, which grows on and damages **local tissue**. The other is **myonecrosis**, inoculated with trauma into muscle and secretes exotoxins that destroy **adjacent muscle**.*

C. Perfringens Clinical Findings:

1- From a **contaminated wound** *(e.g. 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.

> ***Note:*** ***Crepitation*** *is the crackling sensation due to the presence of* ***air*** *(gas) in the* ***subcutaneous*** *tissue.*

2- 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 severe **diarrhea**, usually **without vomiting** or **fever**, in 7–30 hours. The illness lasts only 1–2 days.

Diagnostic Laboratory Tests of C. Perfringens:

- **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 medias is transferred into **milk** which should show a **stormy fermentation** if positive.
- C. perfringens rarely produces **spores** when cultured on agar in the laboratory.
- Final identification rests on **toxin production** and neutralization by specific **anti-toxin**. e.g. **Nagler test**, where an **egg yolk** agar plate is used to detect the **Lecithinase** activity of the **alpha** toxin produced by C. perfringens which breaks down lipoproteins in the plate. The addition of an **anti-toxin** to one half of the plate further confirms.

Treatment and prevention of C. Perfringens:

- 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 is self-limited and requires only **symptomatic care** of fluid and electrolytes replacement.

3- Clostridium Difficile

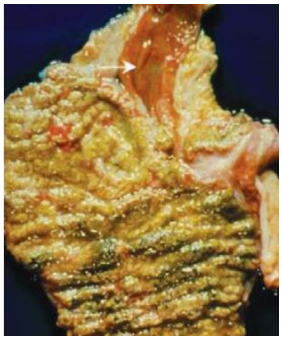
- **Ubiquitous** in the environment and **colonizes the intestine** of **50**% of healthy **neonates** and **4**% of healthy **adults**.
- A **major** cause of **healthcare-associated** infection in patients taking **antibiotics**, where they become at increased risk of developing C. difficile antibiotic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

C. Difficile Pathogenesis:

- Produces two major toxins:
  1- **Toxin A** (enterotoxin): Induces cytokine production with **hypersecretion of fluid**.
  2- **Toxin B** (cytotoxin): Induces **depolymerization** of actin with loss of cytoskeleton.
- **Adhesin factor** and **hyaluronidase** production are also associated virulence factors.
- A **hypervirulent**, hyper-toxin-producing strains are now recognized *(e.g. ribotype 027, 078).*

Disease:

- Clostridium difficile is the pathogen responsible for **antibiotic-associated**:
  1- **Mild-moderate form**: **Diarrhea**.
  2- **Severe form**: **Pseudomembranous colitis** (PMC), characterized by severe diarrhea, abdominal cramping, and fever.



- It follows the use of **broad-spectrum antibiotics** *(such as ampicillin, clindamycin, and the cephalosporins).* These antibiotics can **wipe** out part of the **normal** intestinal flora, allowing the pathogenic C. difficile to **superinfect** the colon.
- Once Clostridium difficile grows in **abundance**, it then releases its **exotoxins**. **Toxin A** causes **diarrhea**, while **Toxin B** is **cytotoxic** to the colonic cells.

Diagnosis of C. Difficile infections (CDI):

- The diagnosis is based on a combination of clinical criteria:
  1- **Diarrhea** *(≥3 loose stools per 24 h for ≥2 days)* with no other recognized causes.
  2- **Toxin A or B** detected in the **stool** *(through ELISA, latex agglutination, and PCR)* or **culture** of **C. difficile** on selective agar.
  3- **Pseudo-membranes** seen in the colon.

- **PMC** is a more advanced, **severe** 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.

> ***Note:*** *PMC is seen as **red inflamed** mucosa and areas of **white exudate** called pseudo-membranes on the surface of the large intestine with **necrosis** of the mucosal surface occurs underneath the pseudo-membranes.*

Treatment and prevention of C. Difficile:

- Because of Clostridium difficile, it becomes very difficile (difficult) to give patients antibiotics since they are the causative agent. Treatment includes:
  1- **Discontinuing** the initial antibiotic.
  2- Oral administration of **metronidazole** or **vancomycin**.
  3- **Fecal transplantation** to restore gut microbiome.
- 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**.
- **Autoclaving** bed pans *(to kill spores).*

> ***Note:*** *The only case where **oral vancomycin** is given is for **CDI**.*

Summary

Spore Forming, Gram-Positive Bacilli:

1- **Bacillus:** Aerobic, generally central spores.

a- B. anthracis → Anthrax, non-motile, capsulated with sticky 'medusa-head' colonies.

b- B. cereus →

- Food poisoning, motile, non-capsulated.
- Produce Lecithinase, hemolysin, cereulide and enterotoxins *(HBE, NHE, cytotoxin K).*
- Self-limited.
- **Diarrheal form**: Long incubation period, from meat, vegetables and sauces. Toxins formed inside the gut. HBE, NHE, cytotoxin K.
- **Vomiting form**: Short incubation period, from rice and cereals. **Pre-formed toxins**. Cereulide.

2- **Clostridium:** Anaerobic, generally subterminal spores.

a- C. botulinum (Flaccid paralysis) →

- Botulinum neurotoxin *"most potent toxin"* that block Ach, has 7 serotypes.
- Canned food and honey.
- Pre-formed toxins in foodborne but not infant botulism.
- **Tx:** Mechanical ventilation and trivalent antitoxin (A, B, E).
- **Dx** of choice: mouse bioassay.

b- C. perfringens (gas gangrene and food poisoning) →

- Stormy fermentation, spores rarely seen in culture, double zone of hemolysis. –
- Forms alpha, epsilon, theta and an enterotoxin.
- **Dx:** Nagler test. **Tx:** Hyperbaric oxygen and Penicillin are lifesaving.

c- C. difficile (PMC) →

- AB associated diseases; diarrhea (mild) or PMC (severe).
- Toxin A (enterotoxin) and Toxin B (cytotoxin).
- **Tx:** Oral **van**comycin and **metro**nidazole.