

بكتريا طيبة 1

Gram Positive Spore Forming Bacilli (Clostridium and Bacillus)



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قسم تقنيات المختبرات الطبية

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الجانب النظري

Lecture No. 9

Theoretical

تدريسي المادة : دكتور معين حسن

(INTRODUCTION)

- **Introduction**
- **Gram Positive Spore Forming Bacilli**
- **Clostridium**
- **Bacillus**

❖ Gram Positive Spore Forming Bacilli (*Clostridium*).

□ Introduction

- Gram-positive, bacilli (rod) shape.
- Obligate anaerobes meaning that oxygen is poisonous to the cells.
- Capable of producing endospores.
- Clostridium endospores have a distinct bowling pin or bottle (Figure) shape, distinguishing them from other bacterial endospores, which are usually ovoid in shape.

1. *Clostridium botulinum*.

A. Virulence factor: include:

1. Produces extremely potent neurotoxins.

- *C. botulinum* is able to produce the neurotoxin only **during sporulation**, which can happen only in **an anaerobic environment**.
- They are **resistant to degradation by enzymes** found in the gastrointestinal tract.
- This allows for ingested toxin to be absorbed from the intestines into the bloodstream.

B. Spectrum of disease and infection

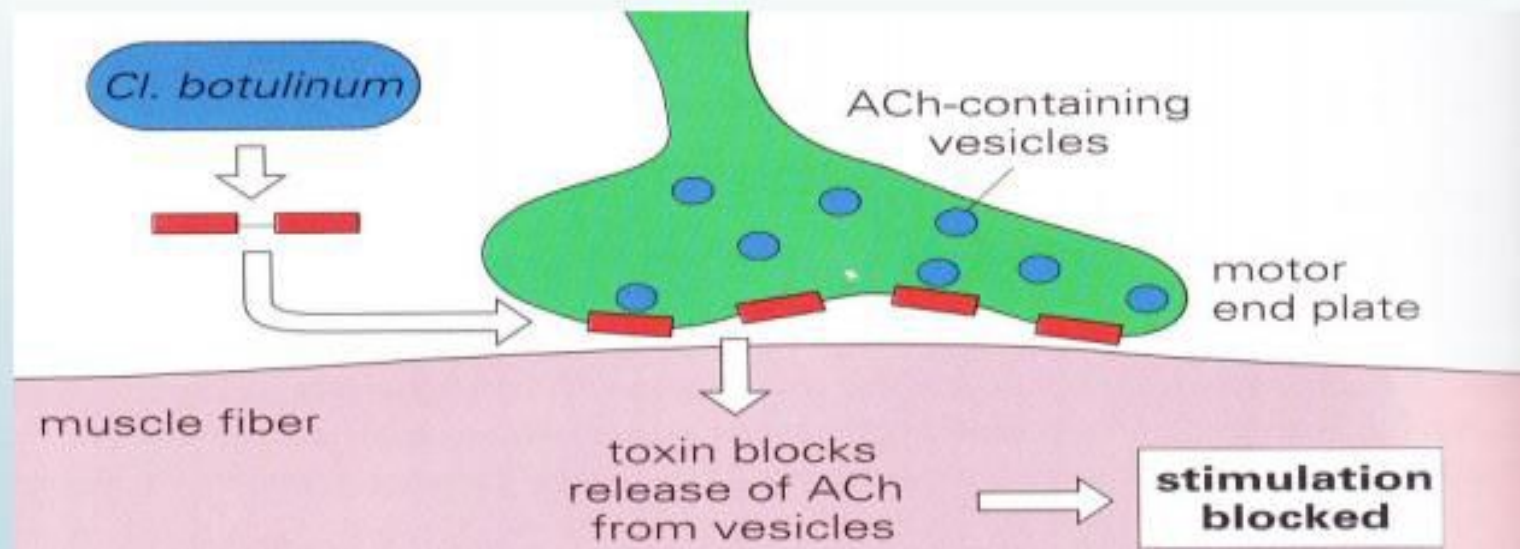
- **Acute food poisoning** that is caused by botulinum toxin produced in food by (*Clostridium botulinum*) and is characterized by:

✓ **Muscle weakness** (flaccid paralytic).

- **Absorption of the toxin** leads to nearly complete paralysis of respiratory and other essential muscle groups.
- The most potent toxin known to humankind, natural or synthetic, with a **lethal dose of 1.3–2.1 (ng/kg)** in humans.

Mode of action of neurotoxin

Botulinum toxin enters the body via the intestine, escaping digestion and crossing the gut wall. The toxin affects peripheral nerve endings at the neuromuscular junction, blocking presynaptic release of acetylcholine (ACh). This prevents muscle contraction, causing flaccid paralysis



Botulism



A 14-year-old with botulism, characterised by weakness of the eye muscles and the drooping eyelids shown in the left image, and dilated and non-moving pupils shown in the right image. This youth was fully conscious.

❖ Pathology:

1. Food borne botulism:

- **Signs and symptoms** of foodborne botulism typically begin between **18 and 36 hours** after the toxin gets into your body.
- But can range from a **few hours to several days**, depending on the **amount** of toxin ingested.

2. Infant botulism:

- If infant botulism is related to food, such as honey, problems generally begin within 18 to 36 hours after the toxin enters the baby's body (can occur when the organism elaborates the toxin after it has colonized the gastrointestinal tract of infants).

3. wound botulism:

- Most people who develop wound botulism inject drugs several times a day, so it's difficult to determine how long it takes for signs and symptoms to develop after the toxin enters the body.
- Most common in people who inject black tar heroin.

Signs of Food-borne and Wound Botulism

- Ventilatory (respiratory) problems
- Eye muscle paresis/paralysis (extra ocular, eyelid)
- Dry mucous membranes in mouth/throat
- Dilated, fixed pupils
- Ataxia
- Hypotension
- Nystagmus
- Decreased to absent deep tendon reflexes

Figure 2. Seventeen-Year-Old Patient With Wound Botulism



A. Patient at rest. Note bilateral mild ptosis, dilated pupils, disconjugate gaze, and symmetric facial muscles.
B. Patient was requested to perform his maximum smile. Note absent smile creases, ptosis, minimally asymmetric smile.

- botulinum is commonly associated with bulging canned food; bulging, misshapen cans are due to an internal increase in pressure caused by gas produced by the bacteria.



2. *Clostridium perfringens*

- (formerly known as *C. welchii*)
- The specific name perfringens is derived from the Latin, per (meaning "through") and frango ("burst"), referring to the disruption of tissue that occurs during gas gangrene.

A. Virulence factor:

1.Alpha toxins is the most important and mediates destruction of host cell membranes; Is a Lecithinase or called (phospholipase – C) that lyses erythrocytes (RBCs), platelets, Leukocytes. and endothelial cell.

▪Alpha toxin mediates:

- ✓massive hemolysis.
- ✓Increased vascular permeability and bleeding (augmented by destruction of platelets).
- ✓Tissue destruction (as found in myonecrosis).
- ✓Myocardial dysfunction (bradycardia , hypotension).

2. **Enterotoxin:** inserts and disrupts membranes of mucosal cells.

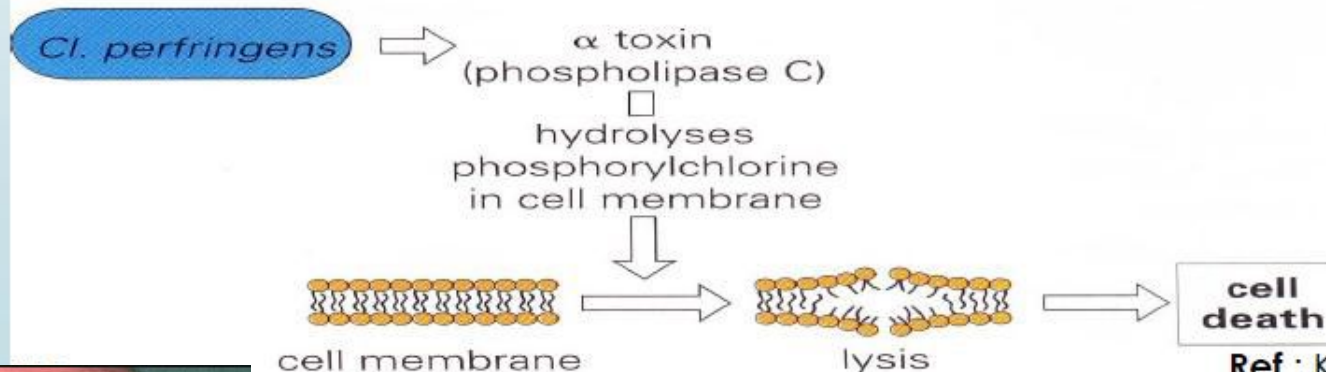
B. Spectrum of disease and infection

- **Gas gangrene;** a life-threatening, toxin-mediated destruction of muscle and other tissues following traumatic introduction of the organism.
- **Food poisoning** caused by **release of the toxin after ingestion of large quantities of organisms.**
- **Disease is usually self-limiting** and benign and is manifested by:
 - ✓ Abdominal cramps.
 - ✓ Diarrhea.
 - ✓ Vomiting.

Mode of action of α -toxin (phospholipase C or lecithinases)

- Toxins may damage or destroy cells and are then known as hemolysis
- Cell membrane can be damaged enzymatically by phospholipases or lecithinases, which destroy the integrity of the cell.

enzymatic lysis



Ref : Kennedy et al., 2009



What are the symptoms of gas gangrene?

▪ Symptoms of gas gangrene often include:

- ✓ Fever
- ✓ air under the skin
- ✓ pain in the area around a wound.
- ✓ swelling un the area around a wound
- ✓ pale skin that quickly turns gray, dark red, purple , or black
- ✓ blister with foul-smelling discharge
- ✓ excessive sweating
- ✓ increase heart rate
- ✓ Vomiting
- ✓ yellow skin and eyes (jaundice) is a late sign.

3. *Clostridium tetani*

A. Virulence factor: include:

1. Tetanospasmin: a neurotoxin exotoxin that disrupts nerve impulses to muscles.

- Tetanospasmin inactivates proteins that regulate release of the inhibitory neurotransmitters' glycine and gamma-aminobutyric (GABA) unregulated excitatory synaptic activity the motor neurons, **resulting in Spastic paralysis**

B. Spectrum of disease and infection

- *C. tetani* (commonly known as lockjaw)
- Organism establishes **a wound infection** and **elaborates the potent toxin** that mediates **generalized muscle spasms**.
- If untreated, spasms continue to be triggered by even minor stimuli, leading to exhaustion and eventually respiratory failure.

TETANUS OR "LOCKJAW"



TETANUS



4. *Clostridium difficile*:

A. Virulence factor: include:

- 1. Exotoxin A:** which is an enterotoxin that is thought to be primarily responsible for the gastrointestinal disease.
- 2. Toxin B:** a cytotoxin, has a less clear role in *C. difficile*.

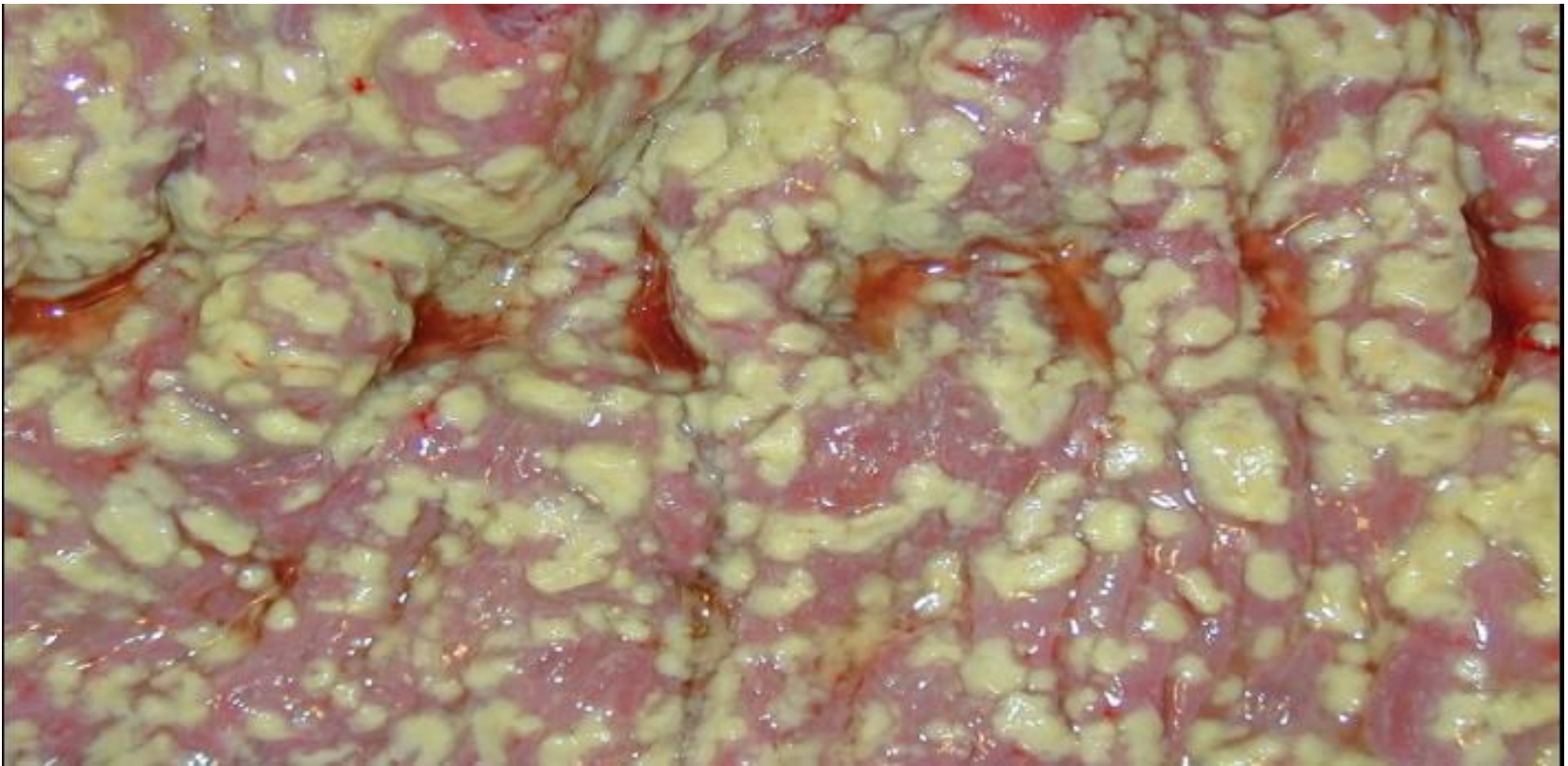
B. Spectrum of disease and infection

- ✓ *C. difficile* infection is spread by bacterial spores found within feces.
- ✓ Organism required diminution of normal flora by the activity of various antimicrobial agents to become established in the gut of hospitalized patients.
- ✓ Once established, **elaboration of toxin (s) results in diarrhea** (i.e., antibiotic-associated diarrhea).
- ✓ Or **potentially life-threatening inflammation if the colon.**

✓ When the surface of the inflamed bowel is overlaid with a "**pseudomembrane**" composed of:

- Necrotic debris, white blood cells, and fibrin.

✓ The disease referred to **as pseudomembranous colitis**.



❖ Treatment:

- In general, the treatment of clostridial infection is **high-dose penicillin G**, to which the organism has remained susceptible.
- *C. welchii* and *C. tetani* respond to **sulfonamides**.
- Clostridia are also susceptible to **tetracyclines**, **carbapenems** (imipenem), **metronidazole**, **vancomycin**, and **chloramphenicol**.

❖ **Bacillus bacteria.**

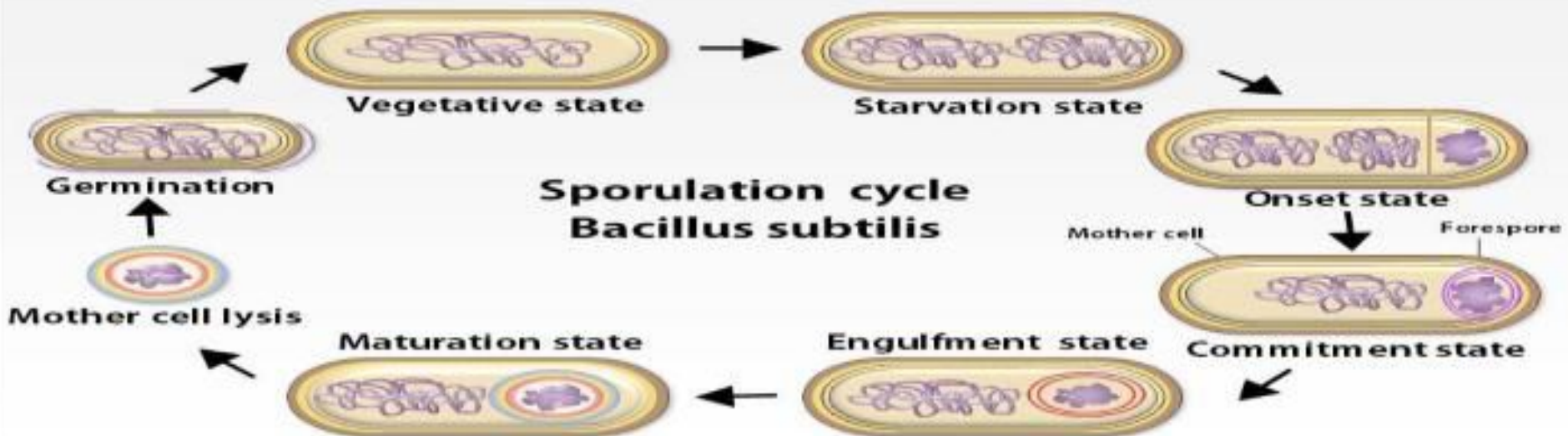
□ **Introduction**

- The genera **Bacillus** and **Clostridium** constitute the family **Bacillaceae**.

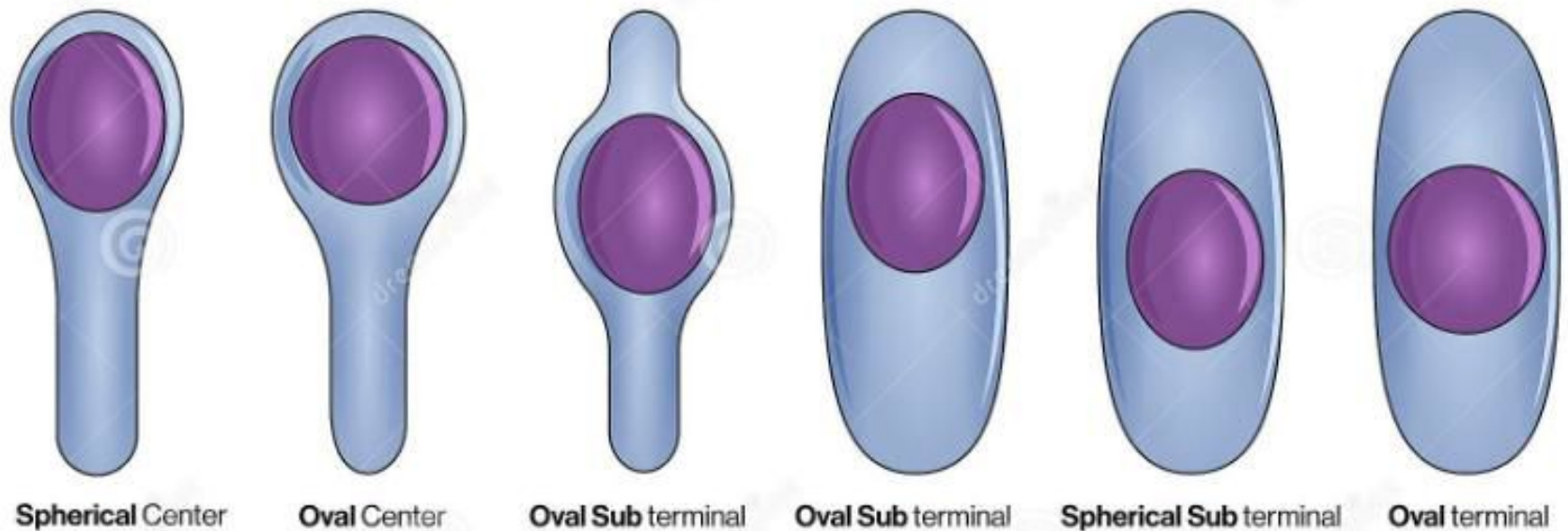
□ **General Characteristics**

- *Bacillus spp.* and related genera **Brevibacillus** and **paenibacillus** all are:
 - ✓ aerobic, Gram positive rods, Are able to survive in adverse environmental condition by formation of oval endospores and can remain in this dormant state for years.
 - ✓ Only one endospore is formed per cell.
- **Endospore formation** is usually triggered by a lack of nutrients, the bacterium divides within its cell wall, and one side then engulfs the other¹⁹.

Formation of a bacterial spore



Positions of spore.



- **Obligate aerobes:** oxygen dependent; or **facultative anaerobes:** having the ability to continue living in the absence of oxygen.

- two *Bacillus* species are medically significant:

✓ *B. anthracis* causes anthrax; and *B. cereus* causes food poisoning.

- *B. anthracis* the most notorious pathogens genus, inhibit the soil.

- Human acquire infections when they are (inoculate with spores, either:

- **Inhalation** during exposure to contaminated animal product such as (hide)

- Or by **traumatic introduction**.

- All **other *Bacillus* spp.** are generally considered to be **opportunistic pathogens** of low virulence and are usually only associated with compromised patients exposed to contaminated materials.

❑ Pathogenesis and virulence factors

A. Pathogenesis of *B. anthracis*

- Is the most **highly virulent species for humans** and is the causative agent on (**anthrax**), of which there are three **(3) form**

1. Cutaneous anthrax: known as **hide-porter's disease** occur at the site of spore presents as a **boil-like skin lesion** that eventually forms an **ulcer with a black center (eschar)**.

- May progress to toxemia and death.



Example Of Cutaneous Infection



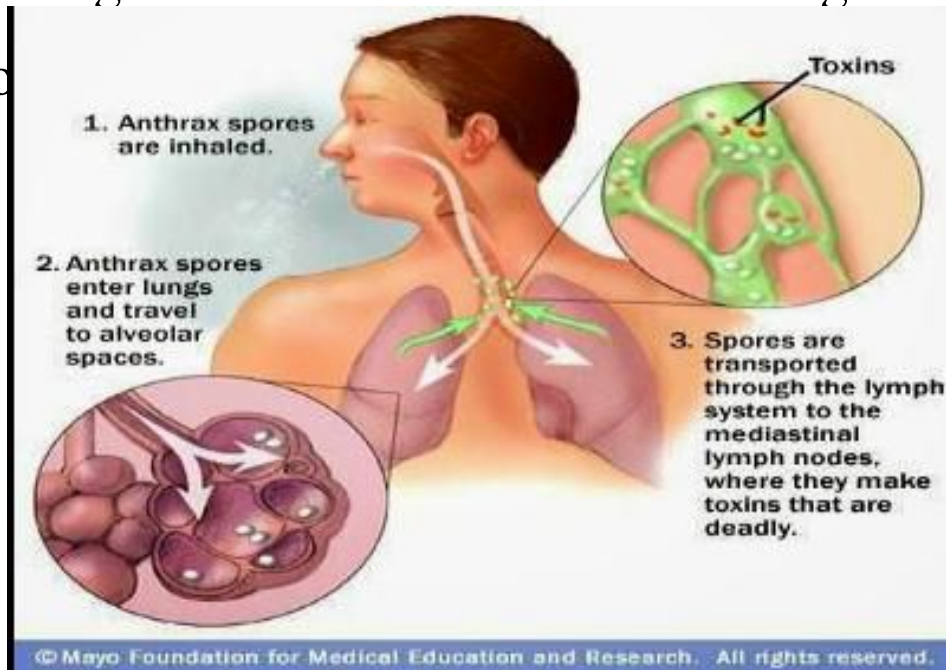
Skin lesion from anthrax



Skin anthrax lesion on the neck

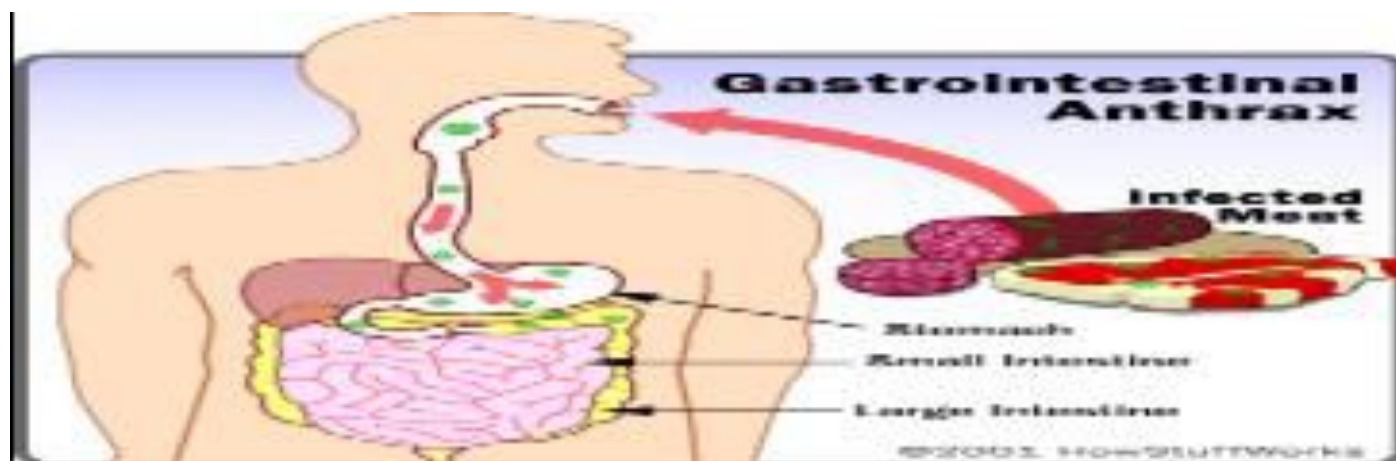
2. Pulmonary anthrax:

- also known as "**wool sorter's" disease**, follows inhalation of spores.
- During the first few days of illness, most people have fever, chills, and fatigue.
- These symptoms may be accompanied by cough, shortness of breath, chest pain, and nausea or vomiting.
- In progressive stage a condition called hemorrhagic mediastinitis, causing bloody fluid to fill the chest cavity, causing shortness of breath.



3. Gastrointestinal anthrax:

- Caused by consuming anthrax-infected meat and is characterized by diarrhea, potentially with blood, abdominal pains.
- Acute inflammation of the intestinal tract, and loss of appetite.
- Occasional vomiting of blood can occur.



❑ Virulence factors of *B. anthrax*

1. Production of antiphagocytic capsule.
2. Potent exotoxins (i.e, edema toxin and lethal toxin) that mediate cell and tissue destruction.

❑ Pathogenesis of *B. cereus*

- Food poisoning of two types:

1. **Diarrheal type**, which is characterized by abdominal pain and watery diarrheal.
2. **Emetic type**, which is manifested by profuse vomiting.

- *B. cereus* is the most commonly encountered species of *Bacillus* opportunistic infections that include:

- Infections of other sites are rare and usually involve
- Intravenous drug abuser or Immunocompromised patients.

❑ **Virulence factors of *B. cereus***

1. Production of enterotoxin.
2. Pyogenic toxin.

❑ **Pathogenesis of other species includes: *B. subtilis*, *B. circulans*, *B. licheniformis* and other.**

- Food poisoning has been associated with some species but is not common.
- These organisms may also be involved in opportunistic infections similar to those described for *B. cereus*.

❖ Laboratory Diagnosis:

- **Samples:** no special consideration is required for specimen collection and transportation.

1. Specimen processing

- Special processing procedure are required for food implicated *B. cerus* in food poisoning outbreaks and animal hides or products, and environmental samples, for the isolation of *B. anthracis*.
- The organisms will be present as spores in these specimens, so initial processing will involve either: Heat or Alcohol shock before plating on solid media.
- The shock will allow only the spore forming bacilli to survive; thus this is an enrichment and selection technique designed to increase the chance for laboratory isolation of these species.

❑ Culture media of Bacillus

- 1. 5 % sheep blood agar.**
- 2. Chocolate agar.**
- 3. Routine blood culture media.**
- 4. Nutrient agar.**
- 5. Not grow on MaConkey.**
- 6. Phenylethyl alcohol agar (PEA).**
- 7. Bicarbonate agar used to induce B. anthracis capsule formation**

QUIZ

Q1/ WHAT IS THE DIFFERENCE BETWEEN BACILLUS AND CLOSTRIDIUM?

CLOSTRIDIUM GROWS IN ANAEROBIC CONDITIONS; BACILLUS GROWS IN AEROBIC CONDITIONS.

CLOSTRIDIUM FORMS BOTTLE-SHAPED ENDOSPORES; BACILLUS FORMS OBLONG ENDOSPORES.

Q2/ WHAT ARE THE CAUSES CANNED FOOD; BULGING

Q3/ WHAT IS THE FUNCTION OF SPORE IN BACTERIA?

A. REPRODUCTION

B. PROTECTION

QUIZ

Q4/ EXAMPLE OF SPORLUATED BACTERIA?

A. BACILLUS AND CLOSTERIDIUM

B. SALMONELLA

TYPHI AND VIBRIO CHOLERA

**Q5/ IN WHICH STAGE SPORULATED BACTERIA PRODUCE
FORESPORE?**

A. STARVATION STATE

B. ONSET STATE

C. COMMITMENT STATE.

Any questions?

*Thank
You*