



Al-Mustaqbal University College
Dept. Medical Lab. Techniques
Diagnostic Microbiology 20/2021
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Lecture-7th.: Bacillus:

After this lecture you would be able to:

- ✓ Describe the cellular and colonial characteristics of *Bacillus anthracis*.
- ✓ Understand the laboratory diagnosis of **anthrax**
- ✓ Describe *B. cereus* which causes food poisoning.

General characteristics of Bacillus:

1. The genus *Bacillus* consists **aerobic** bacilli forming heat resistant **spores**.
2. They are **gram-positive**.
3. *Bacillus anthracis* is **non-motile**.
4. The genus includes **psychrophilic, mesophilic** and **thermophilic** species.

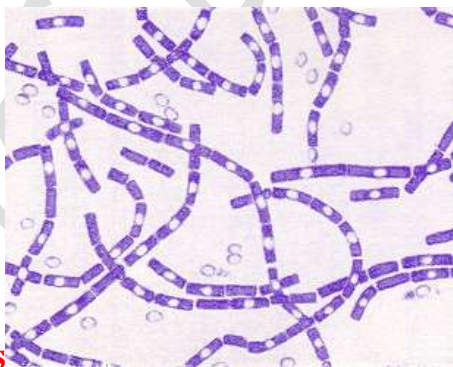
Morphology: 1- *B. anthracis* is **gram-positive, spore forming bacillus**.

2- In cultures, the bacilli are **arranged end to end forming long chains**.

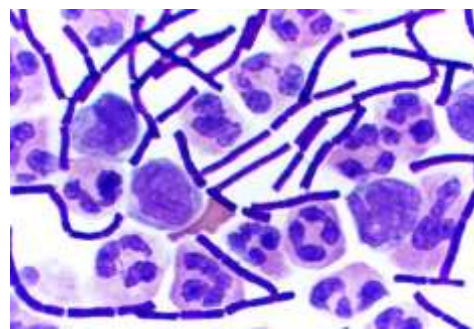
3- The ends of the bacilli are concave and somewhat swollen so that the chain of bacilli presents is 'bamboo stick' appearance.

4- The spore is **oval (ellipsoidal), refractive, central in position and the same diameter as the bacillus, therefore, not swelling the mother cell**. Spores seen as **unstained spaces in Gram-stained bacilli**.

5- The cell is surrounded by a **capsule** which is **polypeptide** in nature, composed of **D- glutamic acid**.



The Spores



The cells

Cultural Characteristics

It is aerobe and facultative anaerobe. Temperature range for growth is 12-45°C (optimum 37°C). Optimum pH for growth is 7.4.

- 1- **On nutrient agar:** On nutrient agar, **colonies are irregularly round, 2-3 mm in diameter, raised, opaque, grayish white, with a frosted glass appearance. The**

edge of the colony is composed of long, interlacing chains of bacilli; resembling 'Medusa head appearance' as in figure below.



Medusa head appearance colonies

2. **On blood agar:** Colonies on horse or sheep blood agar are **non-hemolytic**.
3. **In broth:** Growth develops **silky strands**, a **surface pellicle floccular deposit**.
4. In a gelatin stab, there is growth down the stab line with lateral spikes, longer near the surface, giving an '**inverted fir tree**' appearance with slow liquefaction commencing from the top.
5. **Selective medium:** A selective medium for *B. anthracis* is; (**PLET** medium), consisting of **polymyxin**, **lysozyme**, **ethylene diamine tetra acetic acid (EDTA)** and **Thallos acetate** added to **heart infusion agar**, is used to isolate *B. anthracis* from mixtures containing other spore-bearing bacilli.

Biochemical Reactions: *B. anthracis* ferments **glucose**, **maltose**, **sucrose**, **Trehalose** and **dextrin** with the production of acid without gas. **Nitrates are reduced to nitrites**. **Catalase is positive**. There is a weak **lecithinase** reaction on **egg-yolk agar** which gives a narrow zone of opalescence around the colonies.

Bacillus anthracis: Virulence Factors

- **Capsule** resist phagocytosis; lab presumptive ID by stain or DFA (direct fluorescent antibody) test
- **Exotoxins** complex, coded by plasmid; three genes (three proteins, each alone not toxic)
 - Protective antigen (**PA**) - bind
 - Lethal factor (**LF**) - active
 - Edema factor (**EF**) - active
 - PA combine with LF or EF, binds to host cell receptor, entry into host cell; LF & EF toxic



Anthrax: anthrax is a **zoonotic disease** (it is transmitted between animals and human). It is mainly of animal disease and transmits from animals to human by different ways causing **three types of anthrax disease depending on the site of entry**; either **cutaneous** anthrax (the common and treatable type), **pulmonary** anthrax (**wool's sorter disease**, fatal) or **gastrointestinal** anthrax.

- 1- On the skin = cutaneous anthrax. it is common and treatable.
- 2- Inhalation into the lung = Pulmonary (wool's sorter). It is fatal.
- 3- Ingestion = gastrointestinal anthrax



Cutaneous anthrax on the neck

Cutaneous Anthrax

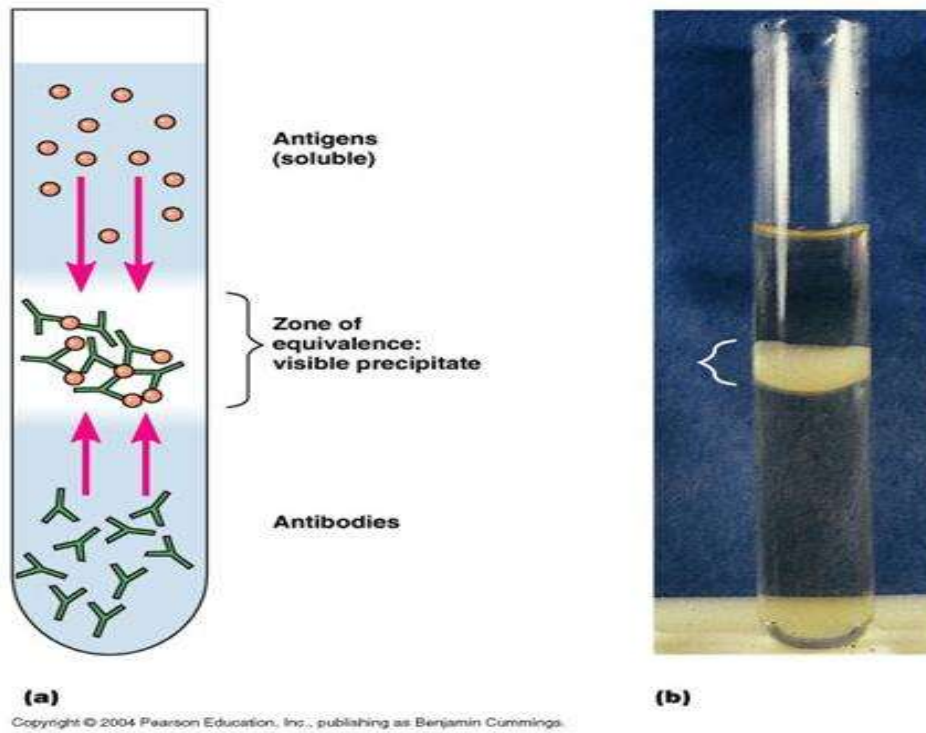
- Most common (95% infections)
- Spores enter exposed skin, germinate, multiply
- Exotoxin released, rapid development of pustule
- Occasionally, MO disseminate - septicemia, death in few days
- Vascular injury - edema, hemorrhage, thrombosis
- Death - respiratory failure, anoxia by toxin on CNS
- Mortality ~20% if untreated



Cutaneous (pustule) anthrax on the arm of man

Diagnosis:

1- **Ascoli test:** it is serological, ring precipitin test using an extract of infected tissue and anthrax antiserum.



2- **McFadyean reaction:** It is special staining reaction, demonstrating a **pink capsule** around a **blue cell** using **polychrome methylene blue stain**.

B. anthracis

McFadyean reaction (polychrome MB stain)



Bacillus cereus: it is a normal inhabitant of the soil, but it can be regularly isolated from foods such as grains and spices. *B. cereus* causes **two types of food-borne intoxications**. One type is characterized by **nausea, vomiting and abdominal cramps** and has an incubation period of **1 to 6 hours**. This is the "**short-incubation**" or **emetic form of the disease caused by (heat-stable emetic toxin)**. The second type causes **abdominal cramps and diarrhea** with an incubation period of **8 to 16 hours**. Diarrhea may be a small volume or profuse and watery. This type is referred to as the "**long-incubation**" or **diarrheal form of the disease caused by the heat-labile diarrhea-genic enterotoxin**. In either type, the illness usually lasts less than 24 hours after onset.

Comparison between *B. anthracis* and *B. cereus*:

B. anthrax vs B. cereus

Sr. No.	Feature	<i>B. anthrax</i>	<i>B. cereus</i>
1	Pathogenicity	Anthrax	Food poisoning
2	Capsule	Poly D-glutamic acid	Absent
3	Motility	Non motile	Motile
4	Source of infection	Spores from animal products	Spores on grains and reheated fried rice
5	Portal of Entry	Skin, Respiratory tract, GIT	GIT
6	Virulence factors	Anthrax toxin, Capsule; inhibits phagocytosis and opsonization	Two types of enterotoxins; one increases the conc. Of cAMP in gut and 2 nd act as super Ag
7	Clinical features	Painless ulcers with black crust, edema, lesion, bacteremia, lymphadenitis	Nausea, vomiting, watery and non bloody diarrhea

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