Spore-Forming Gram-Positive Bacilli (Aerobic Bacteria)

Bacillus species

Objective:

At the end of this lecture you will be able to:

1- Determine the characteristic features of *Bacillus* spp.
2- Recognize the pathogenic species.
3- Point the virulence mechanism to cause infections.
4- Specify the infections that caused by this bacterium.
5- Describe the methods for lab. Diagnosis.

- **General Characteristics of *Bacillus***

1. Large Gram-positive bacilli.
2. Have square ends and are arranged in long chains.
3. Most are saprophytic contaminants or normal flora.
4. *Bacillus anthracis* is the most important member.
5. Aerobic or facultative anaerobic.
6. Produce central endospores.
7. *Bacillus* spp. are ubiquitous
   - Soil, water, and airborne dust

- **Diseases Associated with *Bacillus spp***

1. Anthrax (cutaneous, pulmonary, and gastrointestinal)…. ***Bacillus anthracis***
2. Gastroenteritis, ocular infection, opportunistic infection. ***..Bacillus cereus***
3. Opportunistic infection. ***….Bacillus mycoides***

Bacillus anthracis (Anthrax)

- **Anthrax** is primarily a disease of herbivorous animals (zoonosis). It is an acute disease caused by ***B. anthracis***. Most forms of the disease are lethal, and it affects both humans and other animals.
- ***B. anthracis*** is a large G+ve bacilli found in chains, 1 - 1.2µm in width & (3 - 5µm) in length
- Thiamin growth requirement
- Glutamyl-polypeptide capsule
- Non motile
- Forms oval, centrally located endospores.
- In humans, the infection is usually acquired by the entry of spores through:
    1. **Injured skin (cutaneous anthrax)** (most common).
    2. **Inhalation of spores (pulmonary anthrax "woolsorter's disease")** (deadly).
    3. **Mucous membranes (gastrointestinal anthrax)** (rarely)
- In animals, the portal of entry is the mouth and the gastrointestinal tract.
- Anthrax is an occupational disease (animal's handlers, veterinarians, agricultural workers).
- ***Bacillus anthracis*** is a potential biologic warfare agent.

- **Virulence factors:**

1. The antiphagocytic capsule (poly-D-glutamic acid).
2. Exotoxin - three proteins - protective antigen (PA), edema factor (EF), and lethal factor (LF).
3. Spores which can survive in soil for years.

1- Capsule

- Sticky, gelatinous polymer external to cell wall.
- Made up of D-glutamic acid.
- Only encapsulated B. *anthracis* is virulent.
- Protects against phagocytosis & lysis during vegetative state

2- Toxin

- Protective antigen (PA), edema factor (EF) & lethal factor (LF)
- Protective antigen (PA, 83kDa)
  - *Pag* gene
  - Binds to receptor & helps internalize other 2 proteins
- Edema factor (EF, 89 kDa)
  - *Cya* gene
  - Affects all cells
- Lethal factor (LF, 87 kDa)
  - *Lef* gene
  - More important virulence factor
  - Affects only macrophages
- Make up 50% of proteins in the organism
- Individually non-toxic

  PA+LF → lethal activity

  PA+EF → edema

  EF+LF → inactive

  PA+LF+EF → edema & necrosis; lethal

Pathogenesis

The pathogenicity results from its antiphagocytic capsule as well as from a toxin that causes edemas and tissue necrosis. ***B anthracis*** invades host and produce exotoxin.

The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms. Bacilli spread via lymphatics to the bloodstream, and they multiply freely in the blood and tissues shortly before and after the animal's death.

Anthrax toxin PA binds to specific cell receptors, LF plus PA form lethal toxin, which is a major virulence factor and cause of death in infected animals.

In inhalation anthrax "Wool sorter's disease", the spores from the dust of animal's wool, hair, or hides are inhaled, phagocytosed in the lungs, and transported to the mediastinal lymph nodes, where germination occurs, followed by toxin production.

Clinical Presentation of Anthrax

1- Cutaneous Anthrax

Cutaneous anthrax generally occurs on exposed surfaces of the arms or hands,

- The lesion is called **malignant pustule.**
- Untreated cases progress to bacteremia and death (20%).

2- Pulmonary Anthrax

Woolsorter's disease (pulmonary anthrax is a life – threatening pneumonia).

-The incubation period in inhalation anthrax may be as long as 6 weeks

-Virtually 100% fatal (pneumonic).

Diagnostic Laboratory Tests

1- Specimens: Fluid, pus from local lesion, blood and sputum.

2- Staining methods:

1- Gram stain.

2- Polychrome methylene blue (McFadyean's stain)

3- Immunofluorescence staining.

3- Cultivation:

- ❖ On blood agar media
- ❖ On nutrient agar

Incubation: aerobically at 37°C for 24hrs.

Spores seen after several days of incubation, but not typically in fresh clinical specimens.

4- Demonstration of capsule: (McFadyean's stain).

5- Polymerase chain reaction (PCR).

6- ELISA.

7- Ascoli's test.

- **Treatment & Control**

- Ciprofloxacin is drug of choice.

- **Control measures**

1- Disposal of animal carcasses.

2- Decontamination of animal products.

3- Persons with high occupational risk should be immunized.

- **Vaccine (controversial) (high-risk persons)**

1. Laboratory workers.
2. Active duty military members.
3. Workers that exposed to goat hair, wool and dying cattle.

Bacillus cereus

- Normal inhabitant of the soil.
- *B cereus* Large G+ve bacilli, motile and produces B-hemolytic on blood agar.
- Causes two types of food-borne intoxications.

1. **The emetic form (Intoxication):** is characterized by nausea, vomiting and abdominal cramps ,i.p. 1 to 6 hours. (Associated with fried rice).
2. **The diarrheal form (Food borne Infection):** is manifested primarily by abdominal cramps and diarrhea, i.p. 1–24 hours. (associated with meat dishes and sauces).

Virulence factors:

1. Heat-stable emetic toxin.
2. Heat-labile diarrheagenic enterotoxin.
3. Cytotoxic enzymes

Questions

1- Give the characteristic feature of *Bacillus anthracis*?

2- Which one of the following is an important virulence factor of *Bacillus anthracis*?

(A) Protective antigen
(B) Lipopolysaccharide
(C) Pili
(D) A toxin that inhibits peptide chain elongation factor EF-2
(E) Lecithinase

3- A food commonly associated with *Bacillus cereus* food poisoning is

(A) Fried rice
(B) Baked potato
(C) Hot freshly steamed rice
(D) Green beans
(E) Honey