Bacillus anthracis

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Learning objectives

At the end of the session, the students will be able to

- Describe morphology and antigens
- Describe Pathogenesis & Clinical features
- Choose appropriate lab diagnosis and interpret the results
- Describe prevention and treatment
General Features

- Genus bacillus consists of aerobic bacilli forming heat resistant spores
- Gram-positive but tend to be decolorized easily
- Generally motile with peritrichious flagella (exception *Anthrax bacillus*)
- Most form catalase and produce acid but not gas from glucose
General Features

- The genus includes psychrophilic, mesophilic, and thermophilic species (25 to 75 °c and minimum from 5 °c)
- Spores are ubiquitous, being found in soil, dust, water and air, commonest contaminants in bacteriological culture media
- Pathogenic species - *B. anthracis* and *B. Cereus*
Species

- More than 50 spp. In the genus
- Medically important spp
- *Bacillus anthracis*: Anthrax
- *B. cereus*: food poisoning and opportunistic pathogen
Bacillus anthracis
History

- **1849 - Pollender** – It was the first pathogenic bacterium seen under microscope

- Davaine 1850 - Anthrax was the first communicable disease shown to be transmitted by inoculation of infected blood

- **1876 - Robert Koch** - First bacterium to be isolated in pure culture and shown to possess spores

- **Koch’s postulates** were based on *B. Anthracis*

- **1881 - Louis Pasteur** - Anthrax vaccine was the first live attenuated bacterial vaccine prepared

- Noble prize winner Metchnikoff studied virulent and attenuated strains of *B. anthracis*, in his pioneer work on phagocytosis.
Morphology

- One of the largest of pathogenic bacteria
- Size: 3 to 8 by 1 to 1.3 µm.
- GP, non acid fast, straight, sporing bacilli
- Babboo stick appearance
- Spore is oval, refractile, central in position, and of same diameter
- Capsule composed of d-glutamic acid
- McFadyean reaction: Blood films containing anthrax bacilli with polychrome methylene blue, an amorphous purplish material is noticed around blue bacilli represent capsular material and is characteristics of **B. anthrax**.
Culture

- NA: Irregular, round, 2-3 mm, raised, dull, opaque, grayish white, Medusa head appearance
- BA: Non haemolytic, to narrow zone of haemolysis
- Gelatin: Inverted fir tree appearance
- Selective media; PLET, polymyxin, lysozyme, and EDTA.
Virulence Factors and Pathogenesis

- The pathogenesis depends on two important virulence factors:
  - **Capsule:** (poly d-glutamic acid) interfere with phagocytosis, loss of plasmid which control capsule production leads to loss of virulence
  - **Anthrax Toxin:** Three component protein exotoxin
    1. **Edema factor** - Active fragment
       - Acts as adenylyl cyclase \(\rightarrow\) increases host cell cAMP in host
    2. **Protective antigen** - Binding fragment. Binds to the host cell receptors and facilitates the entry of other fragments into the host cells.
Virulence Factors and Pathogenesis

- Toxin fragments are not toxic individually, but in combination they produce local edema and generalized shock.
- Toxin synthesis is controlled by a plasmid (pX01).
- Loss of plasmid makes the strain avirulent (Basis of original anthrax vaccine prepared by Pasteur)
- **Anthrax Capsule**
  - Polypeptide capsule (poly d-glutamate)
  - Capsule is plasmid (pX02) coded
  - Inhibits complement mediated phagocytosis
Clinical Manifestations

- **Transmission:** based on mode of infection human anthrax presents one of the three ways
  - Cutaneous mode—spores entering through the abraded skin
  - Inhalation of spores
  - Ingestion of carcasses of animals dying of anthrax containing

- **Clinical Types**
  1. Cutaneous anthrax
  2. Pulmonary anthrax
  3. Intestinal anthrax – rare, occurs due to ingestion of spores
Cutaneous v/s Pulmonary Anthrax

<table>
<thead>
<tr>
<th></th>
<th>Cutaneous anthrax</th>
<th>Pulmonary anthrax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Also called as</td>
<td>Hide porter’s disease</td>
<td>Wool sorter’s disease</td>
</tr>
<tr>
<td>Transmission</td>
<td>Cutaneous exposure to spores</td>
<td>Inhalation of spores</td>
</tr>
<tr>
<td>Clinical Features</td>
<td>Malignant pustule</td>
<td>Hemorrhagic pneumonia-</td>
</tr>
<tr>
<td></td>
<td>• Begins as a papule</td>
<td>Bacilli spread by</td>
</tr>
<tr>
<td></td>
<td>→ painless vesicle → coal-black, necrotic eschar</td>
<td>lymphatics or blood</td>
</tr>
<tr>
<td></td>
<td>→ surrounded by non-pitting indurated edema</td>
<td>• Bacteremia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Hemorrhagic mediastinitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Hemorrhagic meningitis</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Self-limiting( 10-20% septicaemis or meningitis)</td>
<td>Fatal</td>
</tr>
<tr>
<td>Bioterrorism</td>
<td>Rarely causes bioterrorism</td>
<td>MC form for bioterrorism</td>
</tr>
</tbody>
</table>
Malignant Pustule

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Animal Anthrax

- Anthrax is primarily a **zoonotic disease**
- **Animals affected** – Herbivores - cattle, sheep and less often horses & pigs
- **Acquired** by ingestion of spores present in soil. Direct spread from animal to animal is rare
- **Presentation** - fatal septicaemia
- **Infecive materials** - Discharges from mouth, nose & rectum. Bacilli sporulate in soil
**Epidemiology**

- **1978-80** RUSSIA, Zimbabwe
- **Animal anthrax**: Progressive global reduction in livestock anthrax due to effective preventive measures
  - Enzootic (endemic) & Epizootic (epidemic) forms
  - Prevalent in Andhra-Tamil Nadu border, foci in Karnataka & West Bengal
- **Human anthrax**
- **Incidence** highest in Africa, central & southern Asia.
  1. **Non-industrial** cases – Agricultural exposure to animals
  2. **Industrial cases** - Infected animal products such as hides, hair, bristles and wools.
Laboratory Diagnosis

- High risk of laboratory acquired infection
- **Specimen Collection**
  - Pus or swab from malignant pustule
  - Sputum in pulmonary anthrax
  - Blood (in septicemia)
  - CSF (in hemorrhagic meningitis)
  - Gastric aspirate, feces or food (in intestinal anthrax)
  - Ear lobes from dead animals.
Diagnosis...Specimen Microscopy

- **Gram staining**
  - Gram-positive, large rectangular rods
  - Spores are usually not seen in clinical samples

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Laboratory Diagnosis...Specimen Microscopy

- McFadyean’s reaction
  - Gurr’s polychrome methylene blue - Capsule appears as amorphous purple material surrounding blue bacilli

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Laboratory Diagnosis...Specimen Microscopy

- **Direct immunofluorescence test (direct-IF)**
- Capsular and cell wall polypeptide antigens detected
- Useful during bioterrorism outbreaks
  - **Ascoli’s thermoprecipitation test**
  - It is a ring precipitation
  - Done when sample is received in putrid form & bacilli are non-viable
CULTURE

- Aerobic, non-fastidious
- Sporulation -25–30°C, distilled water, 2% NaCl, oxalate and oxygen

**Nutrient agar**
- Frosted glass
- Medusa Head Appearance

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CULTURE

- **Blood agar** - dry wrinkled, non-hemolytic
- **Gelatin stab agar** - Inverted fir tree appearance
- **Selective media:**
  - Solid medium with penicillin - string of pearl appearance
  - PLET medium (Polymyxin, lysozyme, EDTA and thallous acetate in heart infusion agar)

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Culture Smear

- **Gram-staining - Bamboo stick appearance**
- **Spores:**
  - Hot malachite green (Ashby’s method)
  - 0.25% sulfuric acid (spores are acid fast)
- **Lipid granules**
  - Sudan black B (Burdon’s method).
Molecular Diagnosis

- PCR with specific primers
  - BA pX01 (encoding protective Ag)
  - BA pX02 (encoding capsular polysaccharide)

- Molecular typing - useful for epidemiological studies
  - MLVA (Multiple locus variable number of tandem repeat analysis)
  - AFLP (Amplified fragment length polymorphisms).
TREATMENT

- **Antibiotic regimen for treatment**
  - Ciprofloxacin/doxycycline + clindamycin, and/or rifampin - 60 days
- **Antibiotics for postexposure prophylaxis**
  - Ciprofloxacin for 60 days + Doxycycline for 60 days or Amoxicillin for 60 days (given if strain is penicillin sensitive).
- **Raxibacumab** - Monoclonal antibody that neutralizes anthrax toxin (protective antigen)
  - For prophylaxis & treatment of inhalational anthrax
Prevention

- **General control measures**
  - Disposal of animal carcasses by burning or by deep burial in lime pits
  - Decontamination (usually by autoclaving) of animal products
  - Protective clothing and gloves for handling potentially infectious materials.
Prevention - Immunoprophylaxis

- **Live Attenuated, Non-capsulated Spore Vaccine (Stern Vaccine)**
  - For animals. Protective for 1 year
  - Not safe for human use.

- **Adsorbed (Alum Precipitated) Toxoid Vaccine**
  - Prepared from the protective antigen
  - Safe & effective for human use
  - Indicated for pre exposure and post exposure prophylaxis
# Anthrax Vaccines

<table>
<thead>
<tr>
<th>Indication</th>
<th>Route</th>
<th>Dosing Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-exposure prophylaxis for persons at high risk of exposure</td>
<td>Intramuscular (0.5 mL/dose)</td>
<td>Primary series: 0, 1, and 6 months Boosters: at 6 and 12 month after primary series and then yearly</td>
</tr>
<tr>
<td>Post-exposure prophylaxis following exposure to suspected or confirmed case</td>
<td>Subcutaneous (0.5 mL/dose)</td>
<td>0, 2, and 4 weeks postexposure combined with antimicrobial therapy</td>
</tr>
</tbody>
</table>
## Anthrax bacilli v/s Anthracoid bacilli

<table>
<thead>
<tr>
<th></th>
<th>Anthrax bacilli</th>
<th>Anthracoid bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Motility</strong></td>
<td>Non motile</td>
<td>Motile</td>
</tr>
<tr>
<td><strong>Capsule</strong></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Bacilli</strong></td>
<td>In long chain</td>
<td>In short chain</td>
</tr>
<tr>
<td><strong>Under low power</strong></td>
<td>Medusa head colony seen</td>
<td>Not seen</td>
</tr>
<tr>
<td><strong>microscope</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood agar</strong></td>
<td>No hemolysis</td>
<td>Hemolytic colony</td>
</tr>
<tr>
<td><strong>Broth</strong></td>
<td>Turbidity absent</td>
<td>Usually turbid</td>
</tr>
</tbody>
</table>
## Anthrax bacilli v/s Anthracoid bacilli

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<thead>
<tr>
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<th>Anthracoid bacilli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicin</td>
<td>Not fermented</td>
<td>Fermented</td>
</tr>
<tr>
<td>Gamma phage</td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>Gelatin stab agar</td>
<td>Inverted fir tree appearance seen. Gelatin liquefaction slow</td>
<td>Not seen Rapid gelatin liquefaction</td>
</tr>
<tr>
<td>Solid medium with penicillin</td>
<td>String of pearls appearance</td>
<td>No growth</td>
</tr>
<tr>
<td>At 45°C</td>
<td>No growth</td>
<td>Usually grows</td>
</tr>
<tr>
<td>Virulence</td>
<td>Pathogenic</td>
<td>Mostly non-pathogenic</td>
</tr>
</tbody>
</table>
**Bacillus cereus**

- Normal habitant of soil
- Widely isolated from vegetables, milk, cereals, spices, meat & poultry
- **Food poisoning**
  - Diarrheal toxin (causes diarrheal type of food poisoning)
  - Emetic toxin (causes emetic type of food poisoning)
- **Ocular disease** - Severe keratitis & panophthalmitis following trauma to the eye
**Bacillus cereus Food Poisoning**

<table>
<thead>
<tr>
<th></th>
<th>Diarrheal type</th>
<th>Emetic type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation period</strong></td>
<td>8-16 hours</td>
<td>1-5 hours</td>
</tr>
<tr>
<td><strong>Toxin</strong></td>
<td>Secreted in intestine (Similar to Clostridium perfringens enterotoxin)</td>
<td>Preformed toxin (formed in diet, similar to S.aureus enterotoxin)</td>
</tr>
<tr>
<td><strong>Food items contaminated</strong></td>
<td>Meat, vegetables, dried beans, cereals</td>
<td>Rice (Chinese fried rice)</td>
</tr>
<tr>
<td><strong>Clinical feature</strong></td>
<td>Diarrhea, fever, rarely nausea</td>
<td>Vomiting, abdominal cramps</td>
</tr>
<tr>
<td><strong>Serotype involved</strong></td>
<td>2,6,8,9,10,12</td>
<td>1,3,5</td>
</tr>
</tbody>
</table>
Laboratory Diagnosis & Treatment

- **Sample** – feces
- **Culture isolation**
  - **MYP**A (mannitol, egg yolk, polymyxin, phenol red and agar)
  - **PE**MBA (polymyxin B, egg yolk, mannitol, bromothymol blue, agar)
- Motile, non-capsulated & not susceptible to gamma phage
**Treatment of *Bacillus cereus***

- Susceptible to clindamycin, erythromycin, vancomycin, aminoglycosides and tetracycline
- Resistant to penicillin (by producing $\beta$-lactamase) and trimethoprim
Bacillus thuringiensis

- Closely related to B. cereus
- Occasionally produce food poisoning
- Used as larvicidal agent for mosquito control
Bacillus spores as Biological controls

- **Geobacilliis stearothermophilus**
  - Autoclave, H2O2 gas plasma sterilization & liquid acetic acid sterilizer

- **Bacillus atropheus**
  - Ethylene oxide sterilizer and dry heat sterilization
MCQs

1. Gram-stain morphology of *Bacillus anthracis* is:
   a. Tennis racket appearance
   b. Drum stick appearance
   c. Bamboo stick appearance
   d. Spectacle glass appearance
2. “Malignant pustule” is a term used for:

a. An infected malignant melanoma
b. A carbuncle
c. A rapidly spreading rodent ulcer
d. Anthrax of the skin
3. Incubation period for *B. cereus* food poisoning following consumption of contaminated fried rice:

a. 1–6 hours
b. 8–16 hours
c. 24 hours
d. >24 hours