Bacillus anthracis

Dr. R.K.Kalyan
Professor
Microbiology

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 peritrichous 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 pgagocytosis.

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 phagocytosi, 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 →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.
3. **Lethal factor** - Causes cell death - Acts by cleaving host cell MAPK (mitogen-activated protein kinases).

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

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

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
- **Infective materials** - Discharges from mouth, nose & rectum. Bacilli sporulate in soil

Epidemiology

- **1978-80** RUSSIA, Zimbabwe
- **Animal anthrax: P**rogressive 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.

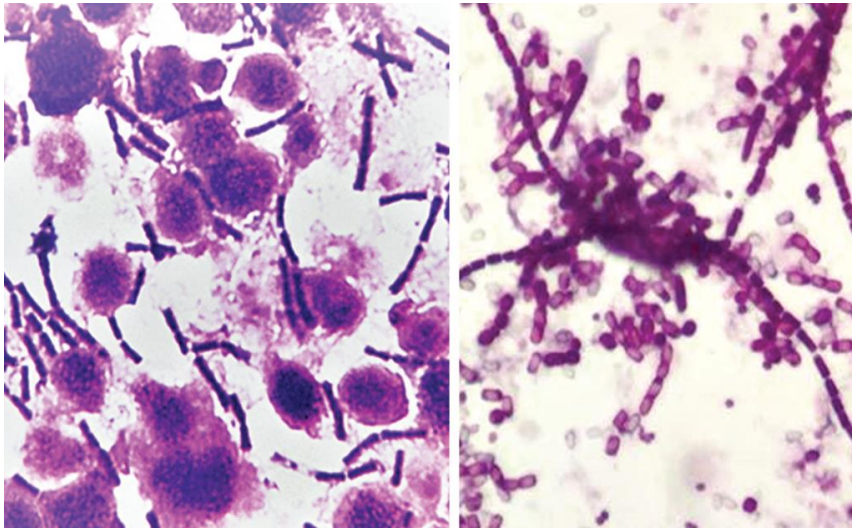
Laboratory Diagnosis

- 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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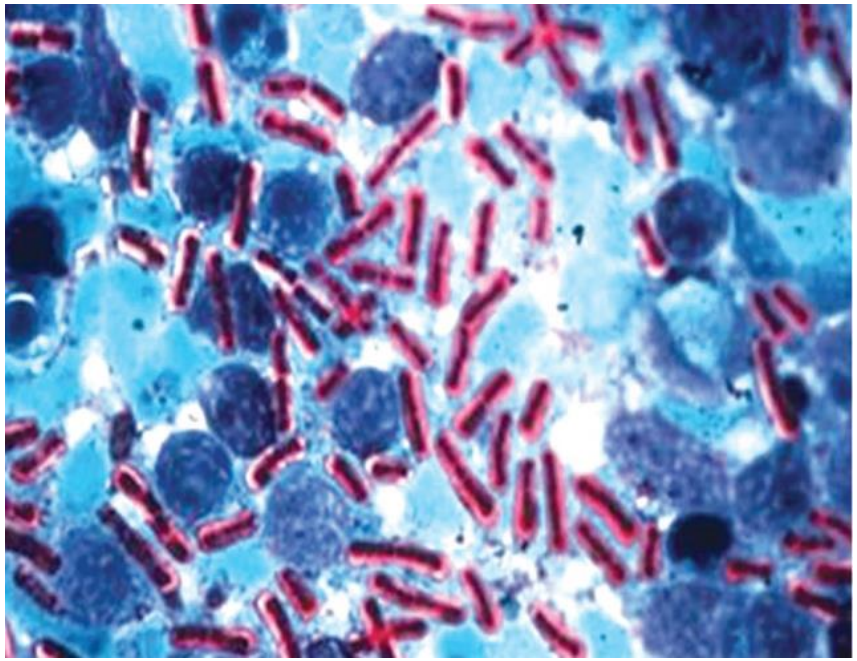
Laboratory Diagnosis…Specimen Microscopy

- **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

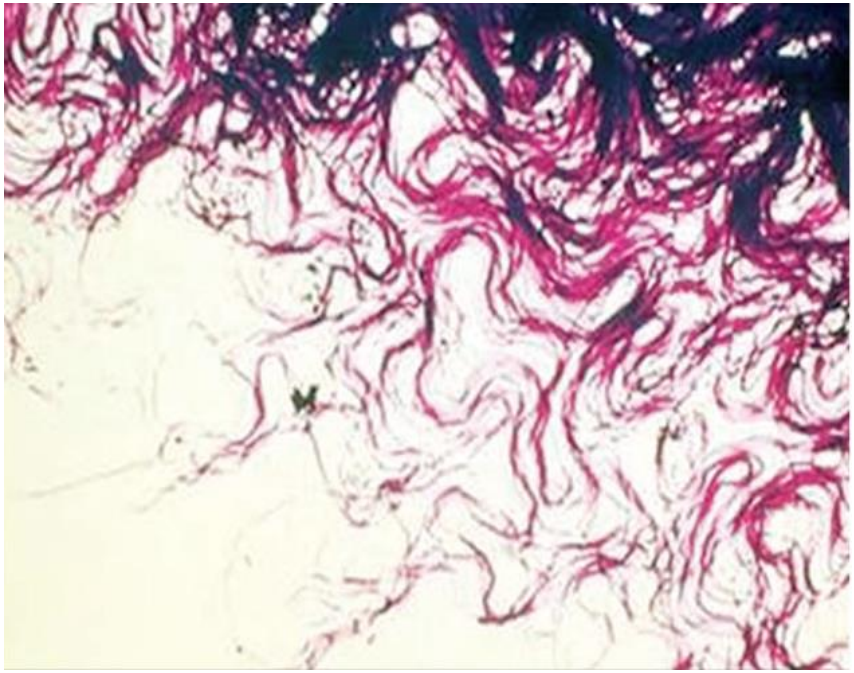
- **Direct immunofluorescence test (direct-IF)**
- Capsular and cell wall polypeptide antigens detected
- Useful during bioterrorism outbreaks
- **Ascoli's thermoprecipitation test**
- It is a ring precipitation
- Done when sample is received in putrid form & bacilli are non-viable

CULTURE

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

– Frosted glass

- Medusa Head Appearance



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)

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

Indication	Route	Dosing Schedule
Pre-exposure prophylaxis for persons at high risk of exposure	Intramuscular (0.5 mL/dose)	Primary series: 0,1, and 6 months Boosters: at 6 and 12 month after primary series and then yearly
Post-exposure prophylaxis following exposure to suspected or confirmed case	Subcutaneous (0.5 mL/dose)	0, 2, and 4 weeks postexposure combined with antimicrobial therapy

Anthrax bacilli v/s Anthracoid bacilli

	Anthrax bacilli	Anthracoid bacilli
Motility	Non motile	Motile
Capsule	Present	Absent
Bacilli	In long chain	In short chain
Under low power microscope	Medusa head colony seen	Not seen
Blood agar	No hemolysis	Hemolytic colony
Broth	Turbidity absent	Usually turbid

Anthrax bacilli v/s Anthracoid bacilli

	Anthrax bacilli	Anthracoid bacilli
Salicin	Not fermented	Fermented
Gamma phage	Susceptible	Resistant
Gelatin stab agar	Inverted fir tree appearance seen. Gelatin liquefaction slow	Not seen Rapid gelatin liquefaction
Solid medium with penicillin	String of pearls appearance	No growth
At 45^0C	No growth	Usually grows
Virulence	Pathogenic	Mostly non-pathogenic

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

B.cereus	Diarrheal type	Emetic type
Incubation period	8-16 hours	1-5 hours
Toxin	Secreted in intestine (Similar to Clostridium perfringens enterotoxin)	Preformed toxin (formed in diet, similar to S.aureus enterotoxin)
	Heat labile	Heat stable
Food items contaminated	Meat, vegetables, dried beans, cereals	Rice (Chinese fried rice)
Clinical feature	Diarrhea, fever , rarely nausea	Vomiting, abdominal cramps
Serotype involved	2,6,8,9,10,12	1,3,5

Laboratory Diagnosis & Treatment

- **Sample** – feces
- ***Culture isolation***
- **MYPA** (mannitol, egg yolk, polymyxin, phenol red and agar)
- **PEMBA** (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 β-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

- **Geobacillius 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

HAVE A NICE DAY