Gram – Positive Rods

Spore – Forming Rods – Aerobic Rods

Bacillus

Species of the genus *Bacillus* are G^+ rods, form endospore, large aerobic rods, rods arrangement in chains. Most members of this genus saprophytic organisms prevalent in soil, water, and air and on vegetation, and in the medical laboratory as airborne contaminants, such as: *B. subtilis* is opportunistic, *B. cereus* causes food poisoning and *B. anthracis* causes anthrax .

A- *Bacillus anthracis*

It's the major important pathogenic bacilli which is occur in goats, sheep, cattle, horses or other animals (rats). Human become infected incidentally contact with infected animals or their products.

Epidemiology

Anthrax is an **enzootic disease** of worldwide occurrence. (an enzootic disease is endemic to a population of animals, compared to an epizootic disease, which attacks a large number of animals at the same time, similar to a human epidemic). Anthrax affects domestic herbivores – sheep, goats and horses and transmitted to humans by contact with infected animal products or contaminated dust (Figure 1).

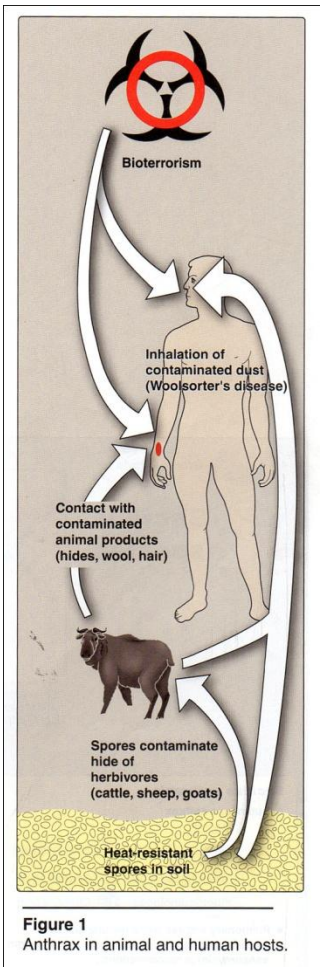

Figure 1
Anthrax in animal and human hosts.

Infection is initiated by the subcutaneous inoculation of spores through incidental skin abrasions. Less frequently, the inhalation of spore–laden dust causes a pulmonary anthrax(**Woolsorter's disease**).

B. anthracis spores may remain viable for many years in contaminated pastures, or bones, wool, hair, hides or other animal materials. These spores are highly resistant to physical and chemical agents. In U.S. a veterinary vaccine in widespread use makes domestic animal sources of the disease quite rare.

Morphology

B. anthracis is G+ rod, large, aerobic, nonmotile ,**have square ends and are arranged in long chains**. Spore is = oval, located in the center of the bacilli and may remain viable on animal products or in the soil for years.

The virulent strains are capsulated (which is antiphagocytic and it's gene is on a plasmid),while avirulent strains are non-capsulated.

Cultural Characters:

It's aerobic grow in 12-45 C° but the optimum temp. for growth is 37 C°. Spores are formed under aerobic conditions at 25-30 °C.

Growth in broth shows no turbidity because they are strict aerobic. On solid media, it's colonies are irregular**(medosa head edg**), round, 2-3 mm in diameter, raised, opaque, grayish white frost glass appearance. Virulent capsulated strains form rough colonies, while avirulent strain form smooth colonies.

Hemolytic selective media PLET (Polymix Lysozyme Ethylenediamine Tetraacetic acid) and thallous acetate is added to heart infusion agar which are used to isolate anthracis from other spore forming bacilli (***B. anthracis*** **is γ – hemolytic on Blood agar).**

Biochemical Reactions:

They ferment glucose, maltose and sucrose with acid only, Nitrate +, Catalase +, **gelatin+**(they contain gelatinase enzyme which degrades amino proteins to form energy).

Antigenic Structure:

1- **Capsule** present in virulent strains consist of polypeptide of high molecular weights composed of D-glutamic acid called hapten (Abs against this Ag are not protective).
2- **Somatic polysaccharides** as complex in cell wall (Abs against it are not protective).

3- **Somatic protein** is present in edema fluid of anthrax lesion (Abs against this is protective).

Pathogenesis:

B. anthracis posses:

a- **Capsule**, that is antiphagocytic and is essential for full virulence.

b- **Three Exotoxines** ,3 plasmid – coded exotoxines:

(1) Edema Factor (EF), causes elevation of intracellular cAMP, and is responsible for the severe edema toxin seen in *B. anthracis* infections with protective antigen(PA).

(2) Lethal Factor (LF), is responsible for tissue necrosis.

(3) Protective Antigen (PA) (because of its use in producing protective anthrax vaccines) mediates cell entry of edema factor and lethal toxin ((PA binds to specific cell receptor and following proteolytic activation, it forms a membrane channel that mediates entry of EF & LF)).

LF + PA form lethal toxin which is a major virulence factor and cause of death in infected animals or in experimental animals (like rats).

The infection is acquired by the entry of spores through injured skin (cutaneous anthrax) or mucous membrane (gastrointestinal anthrax) or by inhalation (inhalation anthrax) → spores germinate in the tissue at the site of entry and growth of the vegetative organisms → formation of a gelatinous edema and congestion. Bacilli spread via lymphatics to blood stream and multiply freely in the blood and tissue shortly before or after the animal's death.

Clinical Significance:

a- Cutaneous anthrax

About 95% of human cases of anthrax are cutaneous. It rapidly evolves into a painless, black, severely swollen "malignant pustule". The organisms may invade lymph nodes and then the general circulation, leading to fatal septicemia. Although some cases remain localized and heal, mortality in untreated cutaneous anthrax is about 20%.

b- Pulmonary anthrax (Woolsorter's disease)

It is caused by inhalation of spores, characterized by progressive hemorrhagic lymphadenitis (inflammation of the lymph nodes), and has a mortality rate 100% if left untreated. It infects persons who handle contaminated animal products.

Spores from the dust of wool, hair, hides are inhaled → phagocytosed in the lungs → transported by the lymphatic drainage → to the mediastinal lymph nodes → germination occurs → toxin production development of hemorrhagic mediastinitis and sepsis.

Woolsorter's disease is high fever, respiratory distress, hemorrhage meningitis may occur as a complication, then death usually results.

C- Gastrointestinal anthrax

Is rare and results from eating contamination meat. Severe enteritis with bloody diarrhea occurs and mortality rates are high.

Laboratory identification

Microscopically: smear prepared from fluid or pus from local lesion, blood and sputum, show **blunt-ended large G^+ bacilli that occur single or in pairs or in long chains (Figure 2) nonmotile, capsulated**.The spore are oval centrally located.

McFadyean reaction: When blood films contain anthrax bacilli, they are stained with polychrome methylene blue for few seconds then examined under the microscope, a amorphous purplish material is seen around bacilli, this represents capsular material and it is a characteristic of anthrax bacilli.

Macroscopically**:** They do not sporulate in clinical samples, but do so in culture. On Blood Agar, the colonies are large, gray, non-hemolytic with an irregular border . A direct immunofluorescence assay aids in identification of the organism.

Animal inoculation : A small amount of exudates or isolated culture from infected man is injected subcutaneously in Guinea pigs which will die within 36-48 hr, smears from the heart blood and spleen show typical G^+ bacilli.

Serological test: Extract of infected tissue shows a ring of precipitate, when layered over immune serum, it is called "**Ascoli test**".

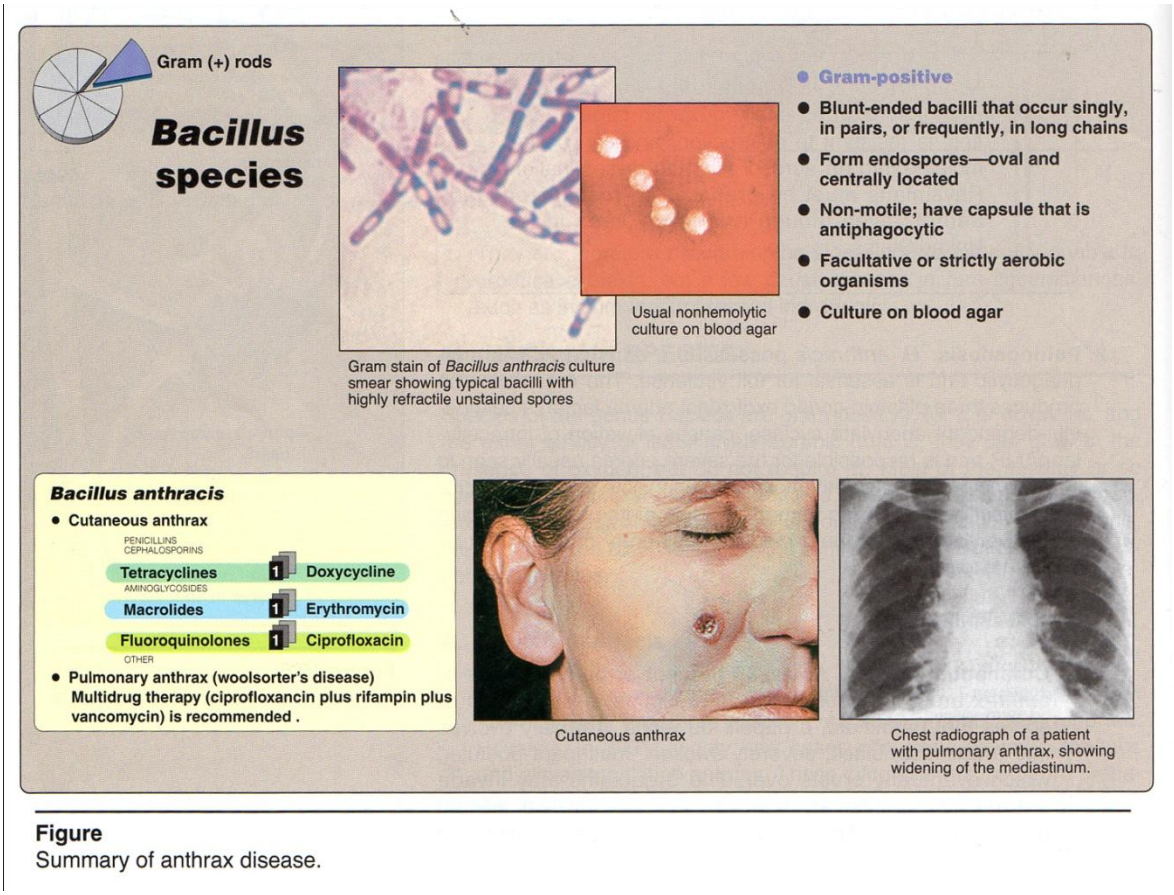

Figure
Summary of anthrax disease.

T**reatment**

They are susceptible to sulfonamide, erythromycin, streptomycin, tetracycline and chloramphenicol.

Cutaneous anthrax responds to doxcycline, ciprofloxacin, or erythromycin (see figure 2). Penicillin is not recommended because of inducible β-lactamase in *B. anthrax*. **In pulmonary anthrax**, multi drug therapy is recommended (ciprofloxacin + rifampin + vancomycin), because of

1- The severity of the disease.

2- The disease is often not diagnosed until late in the course of the illness.

Prevention

1- Vaccine is available for animals and workers in high risk. The incidence of all forms of anthrax is low, particularly the inhalation form, so there no need the vaccine against inhalation anthrax. Vaccine is recommended for goat hair and woolen mill workers, veterinarians, laboratory workers and livestock handlers who are at risk as a result of exposure.
2- Post exposure prophylaxis with ciprofloxacin or doxycycline is recommended.
3- Autoclaving is the most reliable means of decontamination because of the resistance of endospores to chemical disinfectants,.
4- Carcasses of diseased animals should be burned deep in the soil or burned, to prevent the spread of spores.
5- Gas sterilization or radiation may be used to decontaminates hides, wools, and related animals products.

B. *Bacillus subtilis*

B. subtilis is G^+ straight rods, occurring single or in chains, **motile and non capsulated. It grows on blood agar producing β-hemolysis .**

It doesn't produce any toxin, but is **opportunistic pathogens** and may produce serious endophthalmitis (infection in eye ball), right side endocarditis and meningitis.

C. *Bacillus cereus*

Strains of this spps. **produce exotoxin**, and **causes food posining** by means of enterotoxins with either:

1- The emetic type: associated with fried rice, characterized by nausea, vomiting, abdominal cramps, and occasionally diarrhea, begins 1-5 hours after ingestion of the rice.

2- The diarrheal type: associated with meat and sauce, incubation period is 1-24 hours, characterized by abdominal cramps. The enterotoxin may be performed in the food or produced in the intestine.

B. cereus is an important cause of eye infections, severe keratitis, endophthalmitis and panothalmitis, also associated with localized infections and with systemic infections including endocarditis, meningitis, osteomyelitis and pneumonia.

They resist penicillin due to *β-lactmase* . Doxycycline, erythromycin or ciprofloxacin are used as alternatives to penicillin.