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Lecture title: *Bacillus species*

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Summary: Bacillus species

Genus Bacillus:

1. Most Bacillus species are large, Gram-positive, aerobic or facultative anaerobic, endospore-producing rods up to 10 µm in length.

2. A few nonpathogenic species are Gram-negative, and organisms in smears prepared from old cultures decolorize readily.

3. In smears from tissues or cultures, cells occur singly, in pairs, or in long chains.

4. The genus is comprised of more than 50 species with diverse characteristics.

5. Bacillus species are catalase-positive and motile except for *Bacillus anthracis* and *B. mycoides*.

6. Most species are saprophytes with no pathogenic potential. they often contaminate clinical specimens and laboratory media.

7. *B. anthracis* is the most important pathogen in the group.

Usual habitat:

1.Bacillus species are widely distributed in the environment mainly because they produce highly resistant endospores.

2. In soil, endospores of *B. anthracis* can survive for more than 50 years.

3. Some Bacillus species can tolerate extremely adverse conditions such as desiccation and high temperature.

Differentiation of Bacillus species:

The ability to grow aerobically and to produce catalase distinguishes Bacillus species from the Clostridia.

Many species including *B. anthracis* do not produce capsules when grow on laboratory media. Colonial characteristics of Bacillus species which are pathogenic for animals and man:

A- *B. anthracis* colonies:

1. Up to 5 mm in diameter, flat, dry, grayish and with a ground glass appearance after incubation for 48hs.

2. "Medusa head" appearance at low magnification, which is curled outgrowths from edge of the colony.

3. None motile and rarely isolates are weakly hemolytic.

4. Sensitive to penicillin.

5. Negative for lecithinase activity on egg yolk agar.

B- *B. cereus* colonies:

1. Similar to those of *B. anthracis* but are slightly larger with a greenish tinge.

2. The majority of strains produce a wide zone of complete hemolysis around colonies.

3. B. cereus is motile, Haemolytic on sheep blood agar

4. Resistant to penicillin.

5. Strong and rapid lecithinase activity on egg yolk agar.

C- *B. licheniformis* colonies:

1. Dull, rough, wrinkled, and strongly adherent to the agar.

2. Characteristic hair-like outgrowths are produced from streaks of the organisms on agar media.

3. Colonies become brown with age.

4. The name of this species derives from the similarity of it is colonies to lichen.

Clinical infections: *B. anthracis*:

1. Cattle and Sheep: Fatal peracute or acute septicaemic Anthrax.

2. Horses: Subacute anthrax with localized oedema; septicaemia with colic and enteritis sometimes occurs.

3. Pigs: Subacute anthrax with oedematous swelling in the pharyngeal region; an intestinal form with higher mortality is less common.

4. Humans: Three main forms of the disease (Anthrax) occur in man:

a. Skin or Cutaneous anthrax (malignant pustule), is the result of endospores entering abraded skin.

b. Pulmonary anthrax ('wool sorters' disease) follows the inhalation of spores.

c. Intestinal forms of anthrax result from ingestion of infective material.

B. cereus:

1. Cattle: Mastitis (Rare).

2. Humans: Food poisoning, eye infections

B. licheniformis:

1. Cattle and Sheep: sporadic abortion.

2. Also associated with food spoilage.

B. larvae:

Bees: American foulbrood.

B. piliformis:

Laboratory mice, foals and other animals: Tyzzer's disease. An acute fatal infection causing hepatitis, enteritis, and colitis.

Anthrax Disease:

1. Is a severe disease that affects virtually all mammalian species including humans.

2. The disease, which occurs worldwide, is endemic in some countries and defined regions of other countries.

3. Ruminants are highly susceptible, often developing a rapidly fatal septicaemic form of the disease.

4. Pigs and horses are moderately susceptible to infections, while carnivores are comparatively resistant.

5. Birds are totally resistant to infections a characteristic attributed to their relatively high body temperatures.

Epidemiology:

1. Endospore formation is the most important factor in the persistence and spread of anthrax.

2. The endospores of B. anthracis can survive for decades in soil. It has been suggested that, in some geographically defined regions, germination of spores with multiplication of vegetative cells may occur in the soil for short periods at ambient temperatures above 15◦C.

3. Soils in such regions are alkaline, rich in calcium and nitrogen, and have a high moisture content. Such soil conditions also favour spore survival.

4. Outbreaks of anthrax in herbivores can occur when pastures are contaminated by spores originating from buried carcasses.

5. Spores may be brought to the surface by flooding, excavation, subsidence, or by the activity of earthworms. Flooding may also concentrate spores in particular locations.

Pathogenesis and pathogenicity

1. The virulence of B. anthracis derives from the presence of a capsule and the ability to produce a complex toxin. Both virulence factors are encoded by plasmids and are required for disease production.

2. The capsule, composed of poly-D-glutamic acid, inhibits phagocytosis.

3. The complex toxin consists of three antigenic components: protective antigen, oedema factor, and lethal factor. Individually each factor lacks toxic activity in experimental animals, although protective antigen induces antibodies that confer partial immunity.

Clinical signs and pathology

1. The incubation period of anthrax ranges from hours to days.

2. The clinical presentation and pathological changes vary with the species affected, the challenge dose, and the route of infection.

3. In cattle and sheep, the disease is usually septicaemic and rapidly fatal.

4. Although most animals are found dead without premonitory signs, pyrexia with temperatures up to 42°C (108°F),depression, congested mucosae, and petechiae may be observed in antemortem.

5. Animals that survive for more than one day may abort or display subcutaneous oedema and dysentery.

6. In cattle postmortem findings include:

A. Rapid bloating, incomplete rigor mortis.

B. Widespread ecchymotic haemorrhages and oedema.

C. Dark nonclotted blood, and blood-stained fluid in body cavities.

D. An extremely large soft spleen is characteristic of the disease in cattle.

7. Splenomegaly and oedema are less prominent postmortem features in affected sheep, which are reported to be more susceptible than cattle and succumb more rapidly.

Diagnosis

1. Clinical signs:

A. Carcasses of animals that have died from Anthrax are bloated, putrefy rapidly, and do not exhibit rigor mortis.

B. Dark, nonclotted blood may issue from the mouth, nostrils, and anus.

C. The carcasses should not be opened because this will facilitate sporulation with the risk of long-term environmental contamination.

2. Peripheral blood from the tail vein of ruminants or peritoneal fluid from pigs should be collected into a sterile syringe. Cotton wool soaked in 70% alcohol should be applied to the site after collection to minimize leakage of contaminated blood or fluid.

3. Thin smears of blood or fluid, stained with polychrome methylene blue, reveal chains of square-ended, blue staining rods surrounded by pink capsule.

4. Blood and MacConkey agars are inoculated with the suspect specimens and incubated aerobically at 37˚C for 24-48 hours.

5. Identification criteria for isolation:

A- Colonial morphology

B- Microscopic appearance in a Gram-stained smear

C- Absence of growth on MacConkey agar

D- Cultural features and, if necessary, pathogenicity tests in laboratory animals.

E- Biochemical test profile

6. The Ascoli test: is a thermos-precipitation test designed to detect antigens of B. anthracis in biological materials such as hides.

7. Immunological tests like Agar gel immunodiffusion test, complement fixation, ELISA, and Immunofluorescence tests.

8. New molecular diagnostic methods like PCR to amplify specific virulence plasmid markers.

Treatment

Administered early in the course of the disease, high doses of Penicillin G or Oxytetracycline may prove effective.