Bacillus species

Definition:-

Most Bacillus species are large, Gram-positive, endospore producing rods up to 10.0 *µm* in length. **A** few non-pathogenic species are Gram-negative, and organisms in smears prepared from old cultures decolorize readily. In smears from tissues or cultures, cells occur singly, in pairs or in long chains (Fig1)**.**

The genus is comprised of more than 50 species with diverse characteristics. ***Bacillus*** species are catalase-positive, aerobic or facultatively anaerobic and, with the exception of ***Bacillus anthracis*** and ***B. mycoides,*** motile. Most species are saprophytes with no pathogenic potential. However, they often contaminate clinical specimens and laboratory media. ***Bacillus anthracis*** is the most important pathogen in the group.

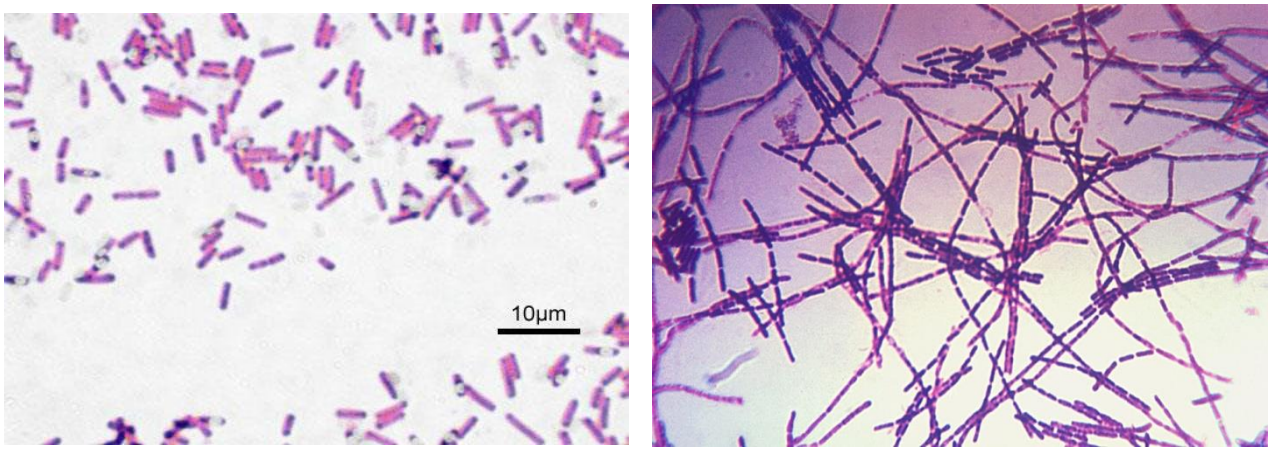


Fig. 1: Bacillus spp

Usual habitat:

Bacillus species are widely distributed in the environment mainly because they produce highly resistant endospores. In soil, endospores of ***B. anthracis*** can survive for more than 50 years. Some ***Bacillus*** species can tolerate extremely adverse conditions such as **desiccation** and **high temperatures**.

Differentiation of *Bacillus* species:

The ability to grow aerobically and to produce catalase distinguishes ***Bacillus*** species from the clostridia, which are also Gram-positive, endospore-forming rods. Differentiation of ***Bacillus*** species is largely based on colonial characteristics and biochemical tests. Many species**,** including ***B. anthracis*** do not produce capsules when grown on laboratory media.

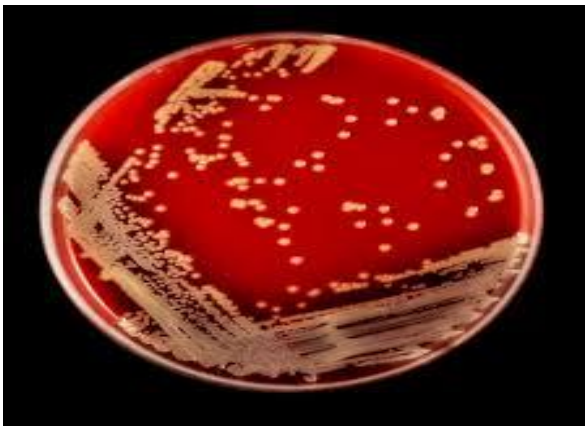
1-Colonial characteristics of ***Bacillus*** species which are pathogenic for animals and man:

a- ***Bacillus anthracis*** colonies are up to 5 **mm** in diameter, flat, dry, greyish and with a 'ground glass' appearance after incubation for 48 hours. At low magnification, curled outgrowths from the edge of the colony impart a characteristic, 'medusa head' appearance. Rarely, isolates are weakly haemolytic.

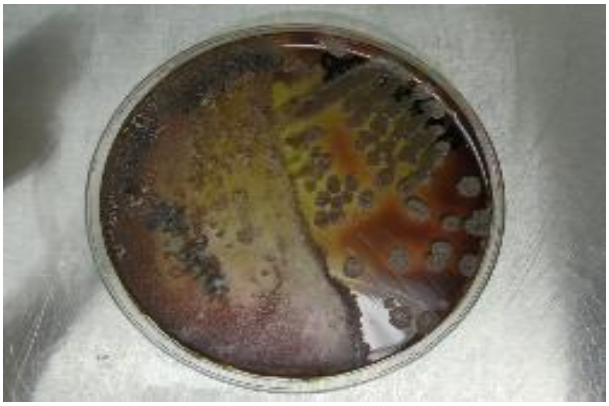
b- ***Bacillus cereus*** colonies are similar to those of ***B. anthracis*** but are slightly larger with a greenish tinge. The majority of strains produce a wide zone of complete haemolysis around the colonies. Because they have some similar characteristics, ***B. anthracis*** and ***B. cereus*** require careful differentiation (Table 1).

c- ***Bacillus licheniformis*** colonies are dull, rough, wrinkled and strongly adherent to the agar. Characteristic hair-like outgrowths are produced from streaks of the organisms on agar media. Colonies become brown with age. The name of this species derives from the similarity of its colonies to lichen.

2-Commercial biochemical test kits for confirming the identification of Bacillus species are available.

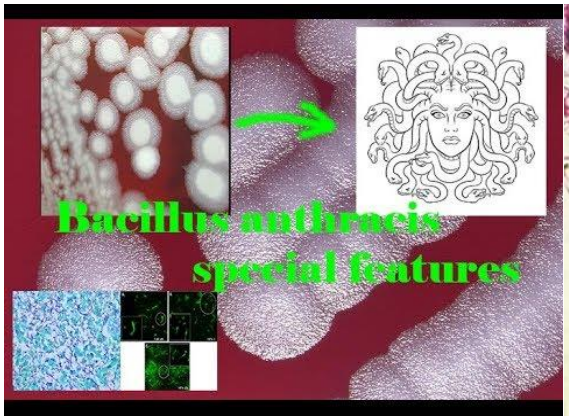


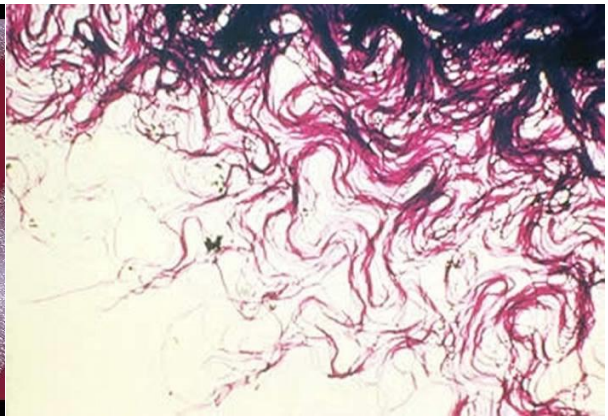
. ***Bacillus cereus***



Bacillus licheniformis

Bacillus licheniformis





Bacillus anthracis **colony (medusa head)**

Fig. 2: Colonial characteristics of ***Bacillus*** species on culture media

Table 1: Differentiating features of ***Bacillus anthracis*** and ***B. cereus.***

Feature	***B. anthracis***	***B. cereus***
Motility	**Non-motile**	**Motile**
Hemolysis on sheep blood agar	**Non-haemolytic**	**Haemolytic blood agar**
Susceptibility to penicillin	**Susceptible**	**Resistant**
Lecithinase activity on egg yolk agar	**Weak and slow**	**Strong and** rapid
Effect of gamma **phage**	**Lysis**	**lysis rarely**

Virulence Factors:

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. The expression of virulence factors is regulated by host temperature and carbon dioxide concentration.

1-**Complex toxin**:-consists of three antigenic components:

a- Protective antigen :binds plasma membrane of target cells cleaved to 2 fragments (by cellular trypsin or proteases) larger fragment is attached to cell surface – binding domain specific receptor mediated endocytosis

b- Edema Factor: (Edema Factor + Protective Ag = Edema toxin) Increased cellular CAMP ⟶ Edema impaired Neutrophil function, Depletes ATP from Macrophages

c- Lethal Factor: (Lethal Factor + Protective Ag = Lethal toxin):-

1- Stimulates Macrophages – TNF alpha and IL – 1 beta – Shock & Death

2- Death due to oxygen depletion, secondary shock, increased vascular permeability, respiratory failure and cardiac failure.

2- **Protein capsule**: - poly d-glutamic acid capsule inhibits phagocytosis (non-capsulated strains – nonpathogenic)

Clinical infections:

The major disease conditions caused by bacteria in this group are listed in Table 2 Anthrax is the most important of these diseases. ***Bacillus licheniformis*** is an emerging pathogen in the group as a cause of abortion in cattle and sheep. ***Bacillus cereus*** is important in human food poisoning and is associated with rare cases of mastitis in cows.

Table 2: Clinical manifestations of diseases caused by *Bacillus anthracis* and other *Bacillus* species.

***Bacillus* species**	**Susceptible animals**	**Clinical manifestations**
***B.* anthracis**	Cattle, sheep	Fatal peracute or acute septicemic anthrax
	Pigs	Subacute anthrax with oedematous swelling in pharyngeal region; an intestinal form with higher mortality is less common
	Horses	Subacute anthrax with localized Oedema: septicaemia with colic and enteritis sometimes occurs
	Humans	Skin, pulmonary and intestinal forms of anthrax are recorded in man periodically
***B.* cereus**	Cattle	Mastitis (rare)
B. licheniformis	Cattle, sheep	Sporadic abortion
B. *larvae*	Bees	American foulbrood

Anthrax

Anthrax is a severe disease which affects virtually all mammalian species including humans. The disease, which occurs worldwide, is endemic in some countries and in defined regions of other countries. Ruminants are highly susceptible, often developing a rapidly fatal septicemic form of the disease. Pigs and horses are moderately susceptible to infection, while carnivores are comparatively resistant. Birds are almost totally resistant to infection, a characteristic attributed to their relatively high body temperatures.

Epidemiology:

Endospore formation is the most important factor in the persistence and spread of anthrax. 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 15^0C. Soils in such regions are alkaline, rich in calcium and nitrogen and have high moisture content. Such soil conditions also favor spore survival. Outbreaks of anthrax in herbivores can occur when pastures are contaminated by spores originating from buried carcasses. 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.

Sporadic outbreaks of the disease have been associated with the importation of contaminated meat-and-bone meal, fertilizers of animal origin and hides. Infection is usually acquired by ingestion of spores and, **less** commonly, by inhalation or through skin abrasions. Although carnivores are comparatively resistant to infection, the ingestion of large numbers of *B. anthracis* from an anthrax carcass can produce disease.

Clinical signs and pathology:

The incubation period of anthrax ranges from hours to days. The clinical presentation and pathological changes vary with the species affected, the challenge dose and the route of infection.

In cattle and sheep the disease is usually septicemic and rapidly fatal. Although most animals are found dead without premonitory signs, pyrexia with temperatures up to **42°C, depression**, congested mucosae and petechiae may be observed antemortem. Animals which survive for more than one day **may** abort or display subcutaneous oedema and dysentery. In cattle, postmortem findings include rapid bloating, incomplete rigor mortis, widespread ecchymosis hemorrhages and oedema, dark unclotted blood and blood-stained fluid in body cavities. **An** extremely large soft spleen is characteristic of the disease in cattle. Splenomegaly and oedema are **less** prominent postmortem features in affected sheep, which are reported to be more susceptible than cattle and succumb more rapidly.

In pigs, infection generally results in oedematous swelling of the throat and head along with regional lymphadenitis. If oedema in the laryngeal region does not interfere with breathing, affected pigs may survive. Intestinal involvement manifests clinically as dysentery due to multifocal, hemorrhagic enteric lesions. Mortality rates may be high.

The clinical course of anthrax in horses is **often** prolonged for several days. Following introduction of spores into abrasions, extensive subcutaneous oedema of the thorax, abdomen or legs may develop. Swelling of the pharynx, similar to that in pigs, has been described. **Less** commonly, colic and dysentery due to severe hemorrhagic enteritis may result from ingestion of spores. If septicaemia occurs, extensive ecchymosis and splenomegaly are found at postmortem.

In dogs, which are rarely affected the course of the disease and pathological changes resemble those observed in affected pigs.

Diagnosis:

1-Carcases of animals which have died from anthrax are bloated, putrify rapidly and do not exhibit rigor mortis. Dark, unclotted blood may issue from the mouth, nostrils and anus. The carcases of such animals 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. Thin smears of blood or fluid, stained with polychrome methylene blue, reveal chains of square-ended, blue-staining rods surrounded by pink capsules .The amount of capsular material diminishes with time after the death of the animal.

3-Blood and MacConkey **agars** are inoculated with the suspect specimens and incubated aerobically at 37°C for 24 to 48 hours.

The identification criteria for isolates are:

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.

4- Biochemical test profile

a-The Ascoli test is a thermo-precipitation test designed to detect antigens of ***B. anthracis*** in biological materials such as hides. Homogenized material is boiled and clarified by filtration. The filtrate is used as the source of antigen in ring precipitation or gel diffusion tests With ***B. anthracis*** antiserum. This test lacks specificity because ***B. anthracis*** shares thermo-stable antigens with other ***Bacillus*** species.

b- Agar **gel** immuno-diffusion, complement fixation, ELISA and immunofluorescence tests have been evaluated for the diagnosis of anthrax, but they are either too insensitive or lack the required specificity for routine use.

3-New molecular diagnostic methods based on the use of PCR to amplify specific virulence plasmid, markers are being developed.

Treatment

If administered early in the course of the disease, high doses of penicillin ***G*** or oxytetracycline **may** prove effectivity.

Control

Suspected cases of anthrax must be reported immediately to appropriate regulatory authorities. Control measures should be designed to take account of the prevalence of disease in a particular country or geographical region.

1-In endemic regions:

a- Annual vaccination, particularly of cattle and sheep, is advisable. The Sterne strain spore vaccine should be given about 1 month before anticipated outbreaks. The spores in this live vaccine convert to non-encapsulated avirulent vegetative organisms.

b- Chemoprophylaxis, employing long-acting penicillin, should be considered when outbreaks threaten valuable livestock.

c- A killed vaccine is available for humans who may be exposed to infection in the course of their work.

2-In non-endemic regions following a disease outbreak:

a- Movement of animals, their waste products, feed and bedding from affected and adjacent premises must be prohibited.

b- Personnel implementing control measures should wear protective clothing and footwear which must be disinfected before leaving the affected farm.

c- Foot-baths containing sporicidal disinfectant *(5%* formalin, or **3%** per acetic acid) should be placed at entrances to affected farms.

c- Contaminated buildings should be sealed and fumigated with formaldehyde before bedding is removed. Following removal of bedding and loose fittings, all drains should be blocked and the building should be sprayed with 5% formalin which should be left to act for at least 10 hours before final washing.

d- Immediate disposal of carcasses, bedding, manure, fodder and other contaminated material is mandatory. Carcasses should be incinerated ***or*** buried deeply away from water courses. Contaminated material and equipment must **be** disinfected with 10% formalin or, if appropriate, incinerated.

f- Scavenger animals should not be allowed access to suspect carcasses and insect activity should be minimized by application of insecticides on and around carcasses.

g- In-contact animals should be isolated and kept under close observation for at least 2 weeks.