# Spore former bacteria

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# Bacillus

- It is saprophytes found in soil, water, air and vegetation
- The most important species are

- Bacillus anthracis
- Bacillus cereus
- Bacillus thuringiensis
- Bacillus subtilis
- Bacillus megaterium

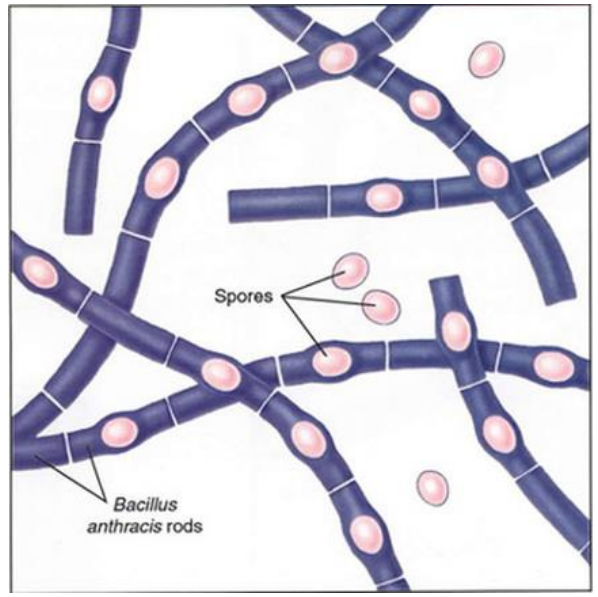
# Scientific classification

## Discovered by Cohn in 1872

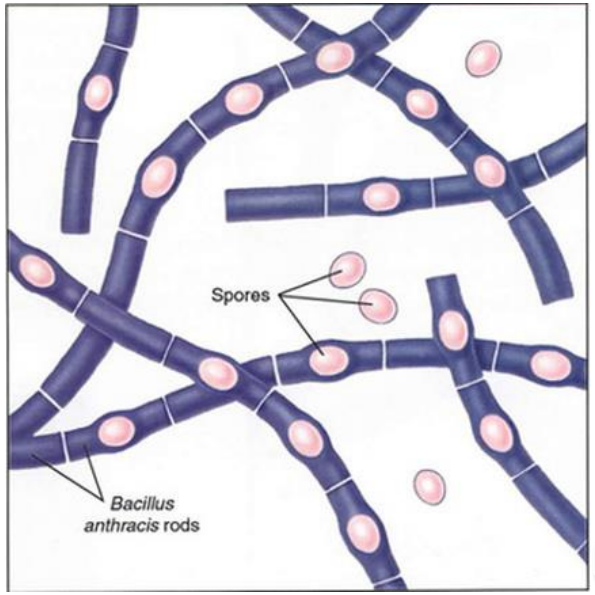
- Kingdom: Bacteria
- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Bacillaceae
- Genus: Bacillus
- **Bacillus anthracis**

# General characteristics

- Gram positive bacteria
- Non motile
- Straight or slightly curved rods and have square ends
- It is one of the largest pathogenic bacteria
- In tissue it is found singly, in pairs or short chains but under artificial culture variable length chains are present

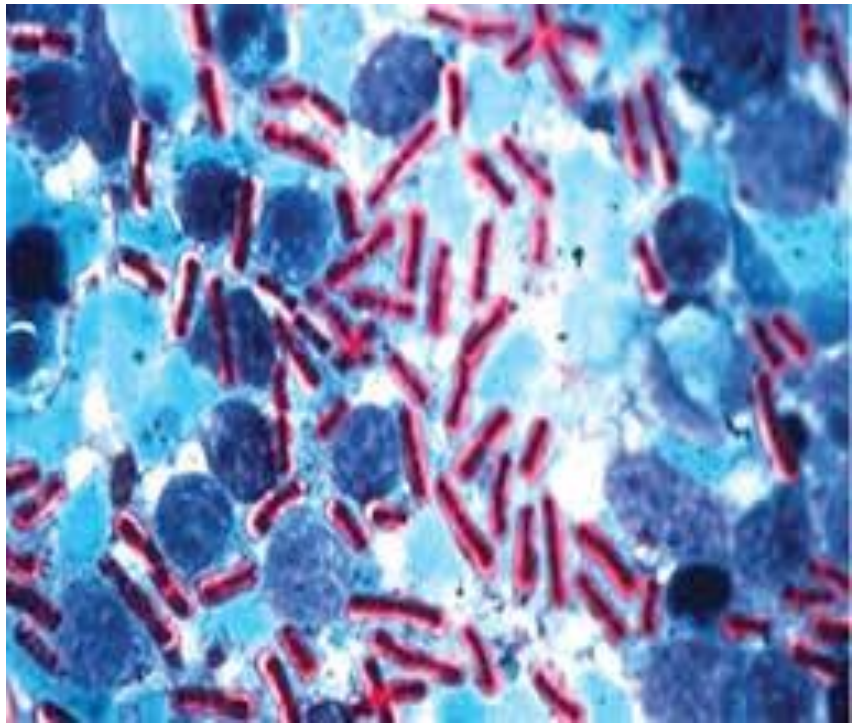


- capsule is formed during growth in the animal body and can be demonstrated in blood and animal tissue.
- The capsular material is a high molecular weight polypeptide of D-glutamic acid
- The spores are found in culture, is soil and material from dead animal , but not in living animal tissue.
- The spores are central, elliptical and not bulging .



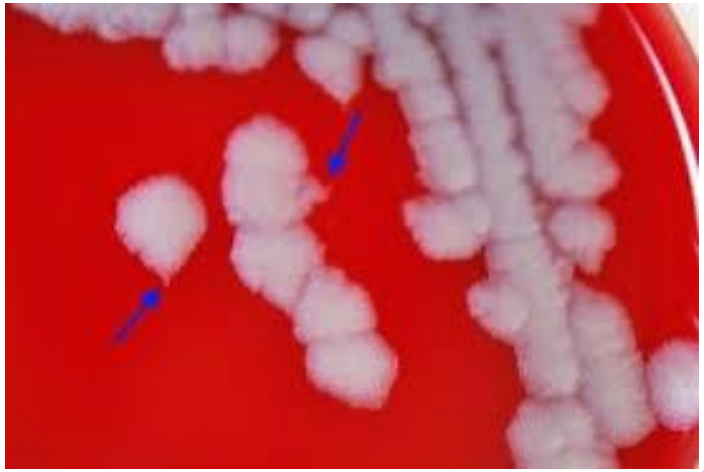
# Mcfadyean reaction

- Veterinary practice for the recognition of anthrax bacilli in blood of infected animals.
- The blood film is dried and passed rapidly three time through the flame and then stained with polychrome methylene blue for a few second, washed , dried and examined.
- The presence of an amorphous purplish material around the bacilli representing disintegrate capsules of the organisms is characteristic of the anthrax bacillus .



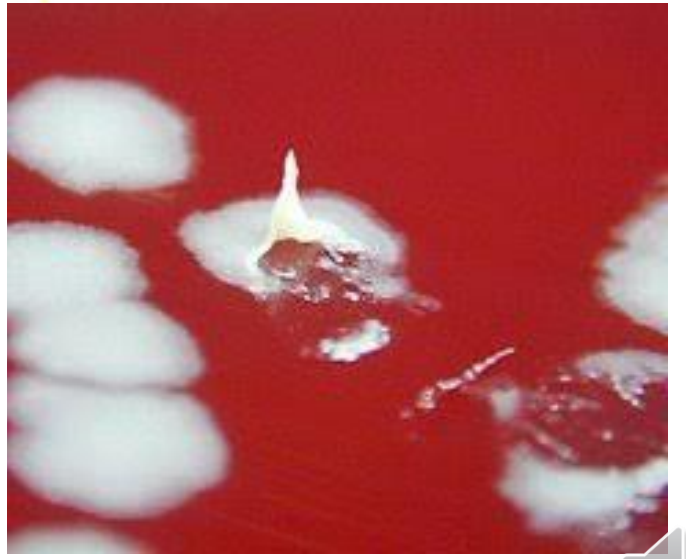
# Cultural characters

- The bacteria is aerobic, facultative an aerobe.
- Optimum growth temperature being 37C at ph 7.5-7.8
- The bacteria grow readily on ordinary culture media and the growth is not much improved by the addition of blood serum or glucose
- It can grows in nutrient broth and on nutrient agar the colonies after 24h



# Cultural characters

- Colonies are about 2-3mm in diameter
- Irregular in outline opaque dull
- Grayish white, surface is irregular giving roughish appearance and emulsifies with difficulty.
- The colonies are medusa head
- On blood agar usually there is no hemolysis around the colonies, thus differentiating anthrax bacillus from saprophytic members which are markedly lytic.

# PLET medium

- A selective medium consisting of

- Polymyxin
- Lysozyme
- EDTA (ethylene diamine tetra acetic acid )
- Thalous acetate

- Added to Brain heart infusion agar, has been devised to isolate Bacillus anthracis from contaminated material.

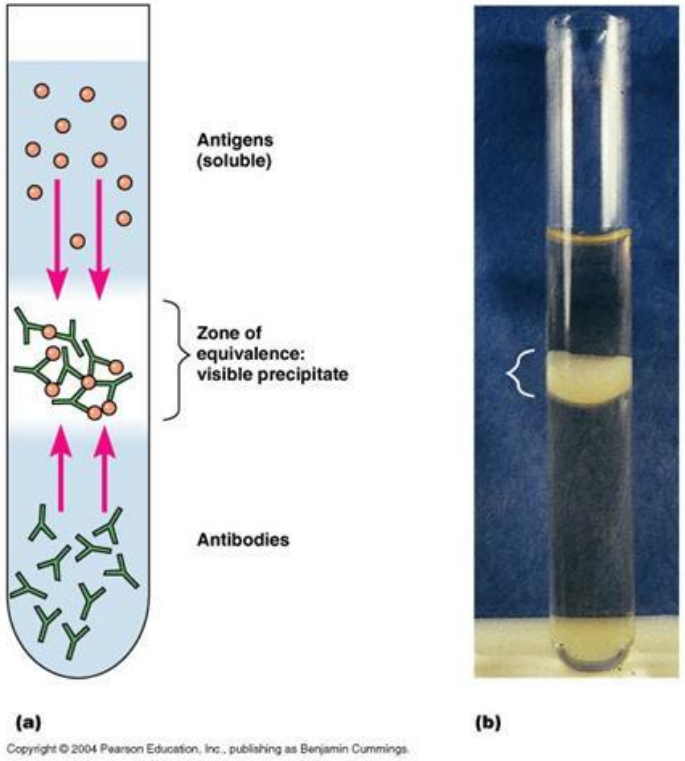
*Bacillus anthracis*

## Biochemical reactions

- These are not of much practical value the identification and classification of the anthrax bacillus
- The bacteria ferments glucose, maltose, sucrose, salicin, dextrin with the production of acid and no gas.
- catalase (+) serological test are not widely employed in the identification of the bacteria except Ascoli precipitation test used in diagnosis of anthrax

# Ascoli precipitin test

- It is used for recognition of anthrax infection in organs and tissues from suspected animals
- About 2 gm of the tissue is boiled, for 5 minunt with 5 ml of normal saline to which acetic and has been added in the proportion of 1:1000
- The fluid is filtered. 0.5 ml of anthrax antiserum is placed in a narrow tube and clear filtrate is carefully on the top of serum. The development of white ring of precipitate at the junction of the two fluids with in 10 minutes at room temperature denotes a positive result .



# Antigenic structure

- Capsular polypeptide
- Somatic polysaccharides
- Protein exotoxin complex

# Protein exotoxin complex

**Anthrax toxin is a complex of three fractions that act synergistically they have been named as :**

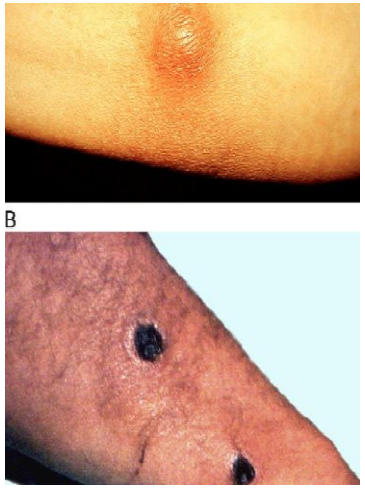
- **The protective antigen ( a protein )**
- **The oedema factor ( EF )**
- **The lethal Factor ( LF)**

**Individually the are not toxic, but the whole complex produces local oedema , shock due to increased capillary permeability .**

# Pathogenesis

In man the infection is acquired from animal sources (herbivores).

three portals of entry have been recognized:



- Through damaged skin
- Inhalation, through mucous membranes through respiratory tract
- Ingestion, spore found in soil on vegetation

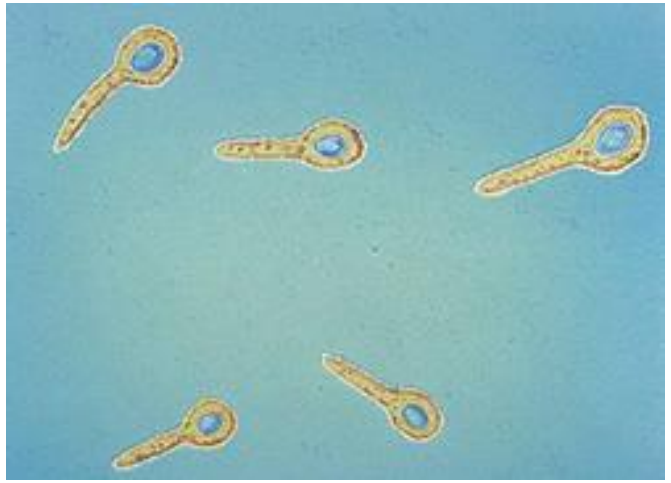
This bacteria cause lesion known as malignant pustule . infection may occur by intestinal route . also it may occur by inhalation of spores carried in the dust of filaments of wool In wool factories when it is referred as wool sorters disease

# Clostridium

- The clostridia are gram positive
- Strict anaerobic
- Spore forming bacilli the pathogenic species produce powerful exotoxins
- Some species are saccharolytic, producing acid and gas from carbohydrates
- Many are proteolytic, the clostridia are widely distributed in nature and are present in soil and in the intestinal tract of man and animals .

# Clostridium

- The spores are usually bulging than the bacillary bodies giving the bacillus a swollen appearance resembling a spindle hence the name clostridium (Kloster meaning the spindle)



- The genus contains organisms responsible for three major diseases of man.

  - the histotoxic or gas gangrene producing clostridia .
  - tetanus producing or Clostridium tetani .
  - botulism producing or Clostridium botulinum

# Scientific classification

**Discovered by Prazmowski in 1880**

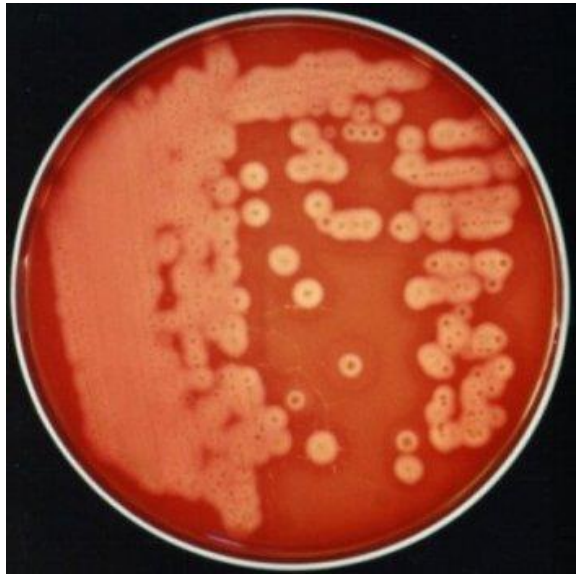
- **Kingdom: Bacteria**
- **Phylum: Firmicutes**
- **Class: Clostridia**
- **Order: Clostridiales**
- **Family: Clostridiaceae**
- **Genus: Clostridium**

# Clostridium perfringens

- In Germany it is called frankel's bacillus, in England it is called Clostridium welchii and in France it is called Clostridium perfringens.
- Gram positive, non motile, anaerobic bacteria spore forming
- Large Capsule in animal tissue
- Occurring in the soil and animal intestine, saccharolytic, produce powerful exotoxins, produce gas gangrene food poisoning and necrotic enteritis in man.
- The bacilli produce oval central or sub terminal spores which are not bulging .

# Cultural characters

## Blood agar



- On this medium , the colonies are round opaque smooth, large, entire edge .
- The colonies are haemolytic, showing complete zone of haemolysis around the colonies, and there may be a wider zone of incomplete haemolysis
- The first zone is due to theta toxin and the second zone of due to alpha toxin

# Cultural characters

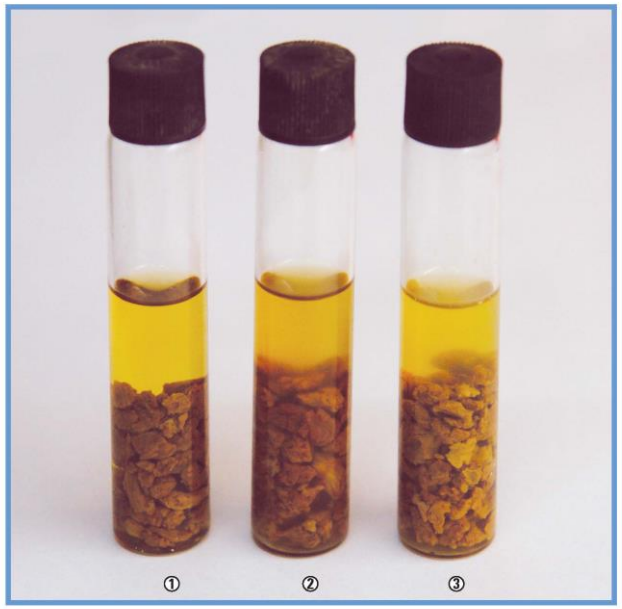
## Willis and Hobb

- This is special selective medium for the isolation clostridia, particularly Clostridium perfringens by the addition of 250 microgram neomycin per ml of the medium.
- Containing lactose, egg yolk and milk agar

# Cultural characters

## Cooked meat medium

- On this medium, the bacteria grow well and meat becomes red and there is no digestion of meat
- Gas production may also be noted this medium
- It contains unsaturated fatty acid which take Up O2
- The reaction being catalyzed by haematin in the meat, and also sulphydryl compounds which bring about a reduced oxidation reduction potential.




Cooked M Medium (R.C. Medium) (M149)
① *Control*
② *Clostridium perfringens ATCC 12924*
③ *Clostridium sporogenes ATCC 11437*

## Vaseline sealed broth

In Vaseline sealed broth, the growth produces uniform turbidity of the broth and due to production of gas, the Vaseline seal is pushed up.

# Biochemical reaction

- Clostridium perfringens is actively saccharolytic and ferments glucose, lactose, sucrose, maltose and starch with the production or acid and gas H2S (+)
- In litmus milk medium acid , clot formation by coagulation casein and marked gas production result in' the production of " stormy clot "
- stormy clot , a reaction which is produced by almost all strains of Clostridium perfringens. this is however not specific this organism .
- Gelatin is liquefied and having little proteolytic activity
- Coagulated serum and egg are not digested
- In cooked meat medium , The meat becomes red and no digestion of meat Occurs .

# Antigenic structure

- The capsular material is polysaccharide and heat stable protiens there are five types of Clostridium refringences designated types A, B, C, D, and E based on the 4 major toxins they produce .

**Toxins and Enzymes :**

All strains of Clostridium refringences , produce powerful exotoxin , with various toxic and enzyme factors, which have haemolytic lethal and necrotizing properties the types of Clostridium refringences Can be ,,differentiated, on the basis of their production of the four major lethal toxin alpha, beta, epsilon and iota .

The main toxins produced by the different types are

- **Type – A strains produce alpha toxin**
- **Type – B strains produce alpha , beta and epsilon**
- **Type – C strains produce alpha and beta toxins**
- **Type – D strains produce alpha and epsilon toxins**
- **Type – E strains produce alpha and iota toxins**

This alpha toxin is produced by all the types so the name *Clos. Per* . is taken here to mean type – A

There are more than 11 toxins and enzymes formed by different strains of *Clos. per.* The amount of these varies With the different types.

**The toxins and enzymes produced are :**

1- alpha toxin –α
2 - Beta toxin –β
3-Epsilontoxin –ε
4 – Iota toxin –ι

**Major toxins**

5-Thetatoxin –0
6-Gamma toxin –γ
7-Delta toxin –δ
8-Eta toxin –η
9-Kappa toxin –κ
10-Lambda toxin –λ
11-Mu toxin –μ
12-Nu toxin –r
13-Bursting factor

**Minor toxins**

# Nagler reaction

It uses observed by Nagler in 1939 that the addition of to filtrate from the growth of Clostridium perfringens to human serum, produces an opalescence and this is known as the Nagler reaction.

- This opalescence is due to the splitting of lipoprotein with the liberation of free fat

Detection for lecithenase

# Human pathogenicity

## Human pathogenicity

**In man, Clostridium perfringens may produce the following lesions and disease :**

- **Gas gangrene-myonecrosis**
- **Food poisoning**
- **Enteritis necroticans**
- **Uterine infections**

# Clostridium tetani

- Gram positive, very strict anaerobe
- Terminal spore giving drum stick appearance
- Motile proteolytic
- Does not ferment common sugar
- Produce very powerful exotoxin
- Responsible to produce tetanus disease

# Clostridium tetani

- The bacteria are straight, slender rod- shaped, the ends are rounded it shows considerable variation in length and may show short or long curved and filamentous forms.
- The bacteria are motile with numerous peritrichate flagella
- The spores are round or oval and terminal, two to four times the diameter of bacillus producing the characteristic drum stick appearance, Its non capsulated

# Cultural characters

## Nutrient agar medium

- After 24-72 hrs incubation an irregularly round, glistening grayish-yellow translucent colonies are formed the central part of the colony may become slightly raised and the edge is filamentous
- In case of motile strain, a fine spreading growth may extend over the surface of the medium whereas non motile strains give discrete colonies .

## Blood agar medium

- Surface colonies are difficult to grow as the growth has a tendency to spread or swarm over the surface of the medium very fine translucent film of growth is produced that is practically invisible except at the filamentous edge
- Swarming character may help in the isolation of organism in mixed cultures containing bacteria that arc less motile than tetanus bacillus the organism may show haemolysis on blood agar due to Tetanolysin .

## Cooked meat medium

Good growth is obtained on this medium and there may occur blackening and digestion of the meat with the production of unpleasant odor

# Biochemical reactions

- Clostridium tetani has proteolytic but no saccharolytic activities
- Thus typical strains of Clostridium tetani do not ferment as carbohydrate
- Litmus milk medium may show no coagulated or delayed clotting
- Gelatin is slowly liquefied
- H2S negative.

# Antigenic structure

- All strains have common O-antigen
- On the basis of flagellar (H) antigen it can be differentiated into ten types designated Roman numericals I to X type VI consists of non flagellated strain type I and III appear to be commonest cause of human infection .

# Toxins

Clostridium tetani produces most powerful exotoxin second only potency to Clostridium botulinum exotoxin. The exotoxins are :

- Tetanolysin
- Tetanospasmin

## Tetanolysin

- It is an O2 labile and heat labile haemolysin
- it is produce by strains of the organism which fail to produce the neurotoxin tetanospasmin
- it is claimed to be leukotoxin necrotizing and cardiotoxic .
- it can be converted to toxoid.

# Tetanospasmin

- It is an essential and major pathogenic constituent which has a selective action on the central nervous system .
- the toxin is protein in nature with a molecular weight of 67,000 it is heat labile 02 stable and it is very good antigen .
- This neurotoxin acts centrally on the nerve cells in the brain and spinal cord rather than on the peripheral nerves the site of action is the nerve ending that have high toxin fixing capacity gangliosides in synaptic membranes are responsible for the binding of tetanus toxin.

## Tetanospasmin

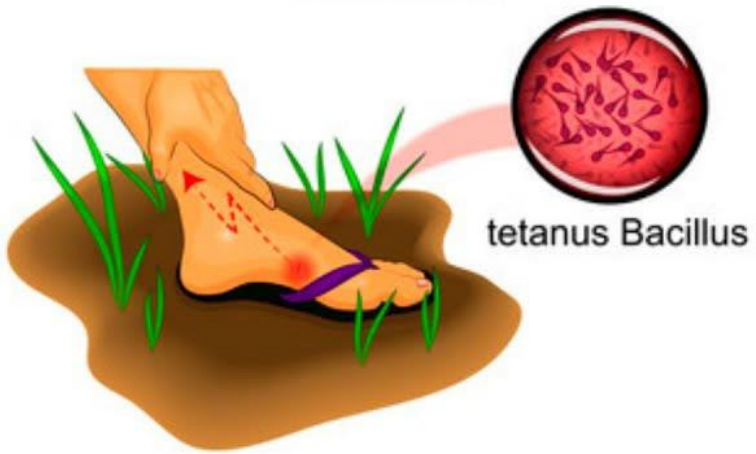
- there is some evidence suggesting that the toxin may act by inhibiting the synthesis and liberation of acetylcholine .
- The toxicity of tetanospasmin is influenced by the route by which it is administered .
- the toxin is destroyed by the action of acid and proteolytic enzymes, by oral route it is inactivated in the stomach and intestinal tract and is with out effect if ingested
- subcutaneous, intramuscular and intravenous injection is more lethal.

# Human pathogenicity

- The organism is normally found in the large intestine of man and animals and also in soil it caused the disease known tetanus.
- Infection may also occur in uterus as in cases of septic abortion .
- Tetanus neonatorum follows infection of the umbilical wound of newborn infants .
- it has been demonstrated that the toxin travels up first along the motor nerve trunks and then up the axis cylinders of the spinal cord and central nervous system and reaches the gangliosides cells of the central nervous system .
- The only part to the central nervous system lies along the axis cylinders of the motor nerve tract.

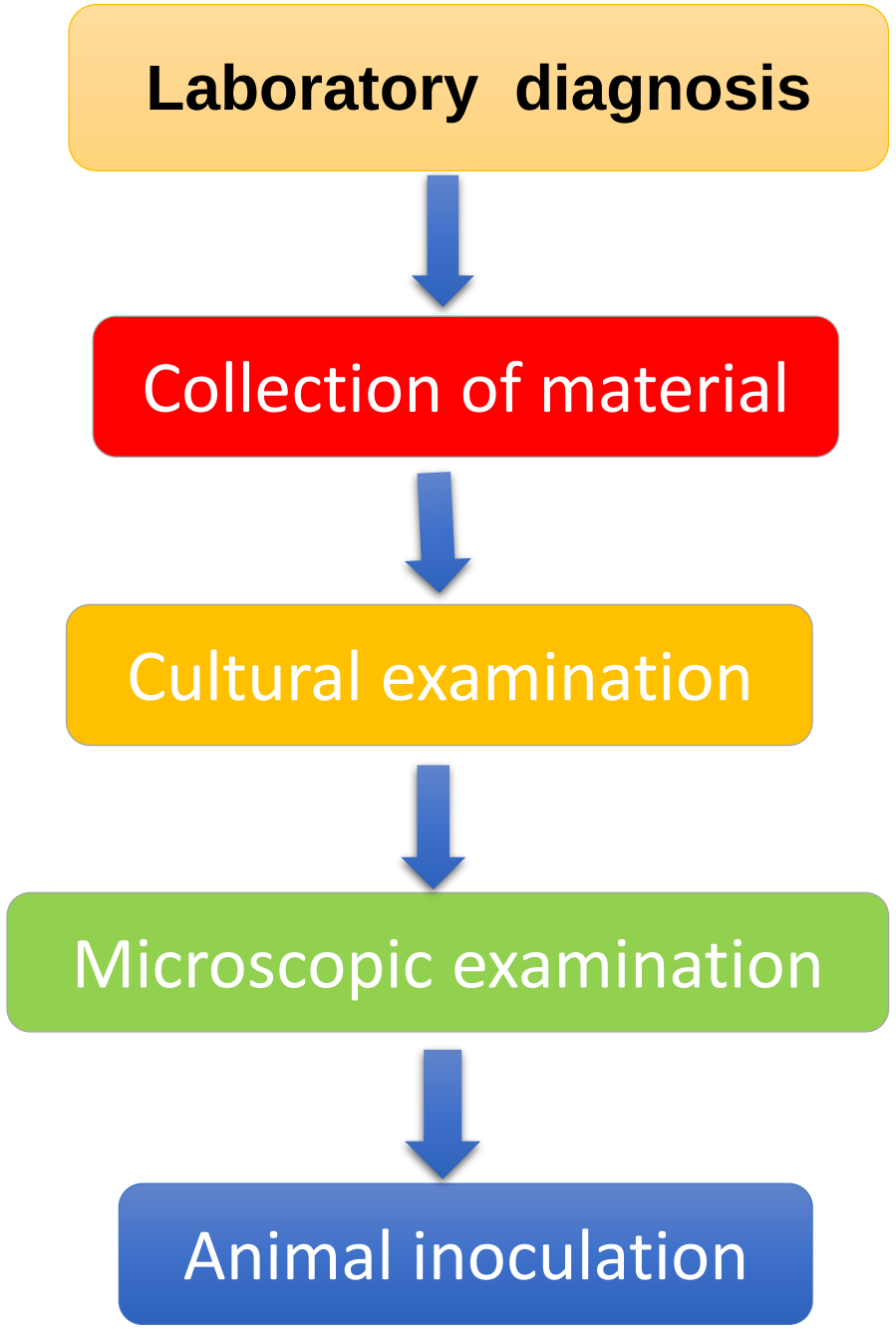
- when the toxin is injected into the hind limb of the animal the toxin is absorbed by the motor nerves and travels up the nerve trunk, thus resulting in ascending tetanus which first affects the muscles of the affected leg and then those of the opposite leg and later muscles of the back and the abdomen, when large dose injected, the total absorption of which by motor nerves is not possible , the toxin may reach the lymph and the passes into the blood stream from which it is absorbed by all the peripheral motor nerves this leads to descending tetanus, which the muscles first affected are those of head and neck producing lock jaw, neck rigidity, affecting the muscles of trunk and back producing opisthotonus.

# TETANUS OR "LOCKJAW"




## Collection of material

- Exudates from the infected wound is aspirated with a sterile pipette, or swabs are rubbed over the wound



# Clostridium botulinum

- Gram positive rod shape
- Strict anaerobe
- Motile spore former the spores are oval, wider than the bacilli bulging and sub-terminal.
- Non capsulated proteolytic
- producing the most powerful exotoxin responsible to produce botulism.

# Cultural character

## Nutrient agar

The colonies after 48 hrs incubation are large glistening translucent irregular with fimbriate border, the center is thicker and slightly brownish, and periphery is thinner and more translucent.

## Blood agar

The colonies are irregular round, large often 48-72 h incubation at 37C. There is zone of beta haemolysis around the colonies except in type G.

## Cooked meat medium

Meat is digested and blackened with putrid odor .

# Biochemical reaction

- The bacteria ferments glucose and maltose with the production of acid and gas .
- proteolytic and slowly liquefy coagulated serum milk casein is digested and meat is also digested and becomes black .
- $H_2S$ is produced by some types .
- strains can be separated in to two groups : the strongly proteolytic (Ovolytic) strains and the less proteolytic (Novolytic) strains
- All strains hydrolyze gelatin .

# Antigenic structure

- This bacteria possess common O antigen .
- On the basis of flagellar antigen it is classified in to seven main types A, B ,C, D, E, F and G. types A B and E are those most commonly associated with botulism in human .

# Toxins

- The toxin of type-A has been isolated as a pure crystalline protein and quantitatively, is probably the most potent substance in nature one micro gram of the purified toxin contains about 200,000 minimal lethal close (MLD) fora 20 gm white mouse .
- The toxin with stand gastric juice and is absorbed intact it is claimed that 15 gm of pure toxin is sufficient to poison the entire human population of the world . The toxin acts at the myoneural junction, apparently by preventing the release of acetylcholine from demyelinated ends of the motor nerves, There is no effect on the peripheral nerves .

# Pathogenesis

- Botulism is thought to be due to absorption from the intestine of toxin performed by the bacilli in preserved food such as ham, sausage, canned meats, vegetable, fish and fish produced .
- botulism has not been associated with fresh foods, cooked or raw.
- The toxin can be absorbed through respiratory mucous membranes beside its absorption by the gut wall.

# Pathogenesis

- After absorption from the gut, toxin can be found in the blood, hence it is absorbed by the peripheral nervous system .
- Toxin acts at myoneural junction and produce death by respiratory paralysis. Unlike tetanus , the central nervous system is not effected and the effect appears to be rather peripheral.

# Laboratory diagnosis

- Isolation of the organism from the baby is not possible, but an attempt should be made to demonstrate the organisms and the toxin in the suspected food
- The material referred to the laboratory for bacteriological examination is usually sample of food and rarely it may be faeces or vomit, which may be subjected to following examinations .

# Laboratory diagnosis

## 1- Smear examination

## 2- Culture examination

- The sample of contaminated food is heated for 30 min at 65c to 80c, to eliminate non sporing bacilli .
- Subsequently the culture may be made under anaerobic condition on solid media and cooked meat broth, by the morphology and toxigenicity the organisms are identified.

# Laboratory diagnosis

## 3- Animal inoculation

- The food sample is well mixed with sterile saline and macerated the clear filtrate often centrifugation is injected intraperitonelly in to guinea pigs in about 2m1 amount.
- In the control group of animal, the extract heated at 100c for 10min, is injected. In a third group, the unheated extract along with different types of antitoxins may be inoculated. No death of animals should occur in the second and third (control) group of animals, whereas, the test animal show manifestations of the disease and die.

# Clostridium difficile

- Causses antibiotic associated diarrhea
- Colitis
- Pseudomembrane colitis on human
- Over growth of bacteria cause this disease that because normal flora disturbed by antibiotic therapy