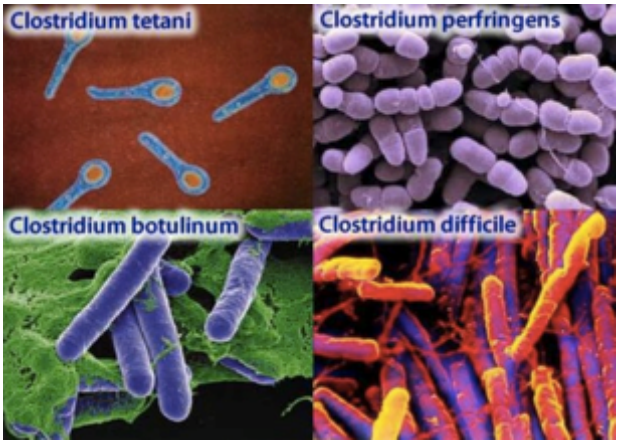
Family:	**Clostridiaceae**
Genus:	***Clostridium***



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 carbohy drates and many are proteolytic , the clostridia are widely distributed in nature and are present in soil and in the intestinal tract of man and animals. 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.

1) The histotoxic or gas gangrene producing clostridia.
2) Tetanus producing or *Clostridium tetani*.
3) Botulism producing or *Clostridium botulinum*

Clostridium perfringens

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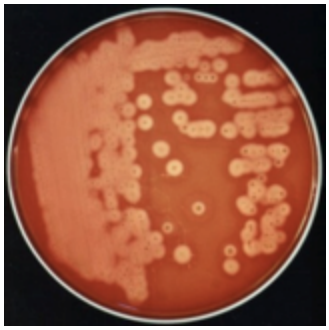
In Germany it is called frankel's bacillus, in England it is called *Clostridium welchii* and in France it is called *Clostridium perfringens*. Gram positive, non motile, anaerobic bacteria spore forming, capsulated in animal tissue, occurring in the soil and animal intestine, saccharolytic , produce powerful exotoxins , produce gas gangrene food poisoning and necrotic enteritis in man . The bacilli produce oval, central or subterminal 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**

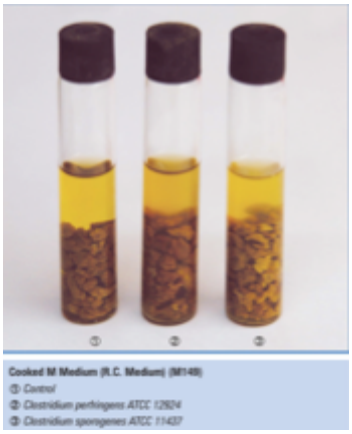


Willis and Hobb:

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

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.



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:

Clos . perfringens is actively saccharoly 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 " , a reaction which is produced by almost all strains of *Clos . 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 .

stormy fermentation

Antigenic structure:

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

Toxins and Enzymes: All strains of *Clos. per. ,* produce powerful exotoxin , with various toxic and enzyme factors, which have hemolytic lethal and necrotizing properties the types of *Clos. per.* 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 12 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 –
2-Beta toxin – **Major toxins**
3-Epsilontoxin–
4 – Iota toxin –

5-Thetatoxin–0
6-Gamma toxin
7-Delta toxin
8-Eta toxin
9-Kappa toxin **Minor toxins**
10-Lambda toxin
11-Mu toxin
12-Nu toxinr
13-Bursting factor

Nagler reaction: It uses observed by Nagler in 1939 that the addition of to filtrate from the growth of *Clos. per* . 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 .**

Human pathogenicity :

In man ,*Clos. perfr.* may produce the following lesions :

a)Gas gangrene — myonecrosis .

b)Food poisoning .

c)Anaerobic cellulitis .

d)Puerperal sepsis .

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(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 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: *Clos. tetani* has proteolytie but no sacccharolytic activites and thus typical strains of *Clos. tetani.* donot ferment as carbohydrate . Litmus milk medium may show no coagulated or delayed clotting, gelatin is slowly liquefied H2S neg.

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 :

Clos. tetani. produces most powerful exotoxin second only potency to *Clos. botulium* exotoxin . the exotoxins are :

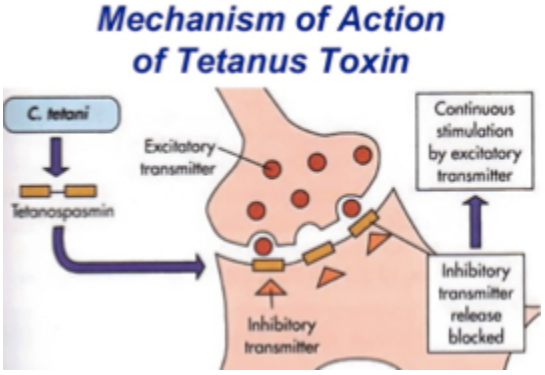
a) Tetanolysin b)Tetanospasmin

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. there is some evidence suggesting that the toxin may act by inhibiting the synthesis and liberation of acetylcholine . The route by which it is administered influences the toxicity of tetanospasmin.** 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.**

Laboratory diagnosis:

The laboratory diagnosis may be considered under the following headings:

1- Collection of material
2- Microscopic examination
3- Cultural examination
4- Animal inoculation

Collection of material: exudates from the infected wound are 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 characters:

Nutrient agar :

After 48 hrs incubation, the colonies 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 37°C.

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:

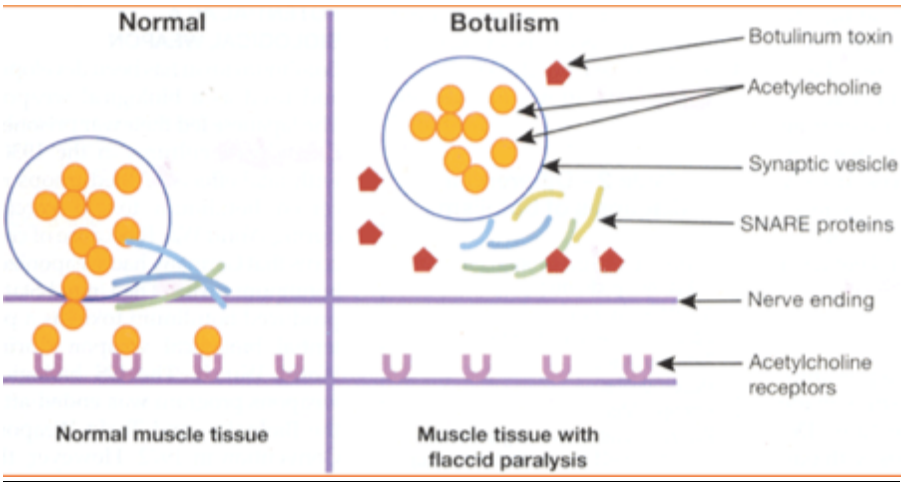
This bacteria possess common O antigen . On the basis of flagellar antigen it is classified in to seven main types A,B ,C,D,E,F and G. types A B and E are those most commonly associated with botulism in human .

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. 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 feces or vomit, which may be subjected to following examinations

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.

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.