

## *Clostridium*

**General characters:** The genus *Clostridium* consists of Gram-positive, anaerobic, spore forming, spindle shaped and highly pleomorphic bacilli. Spores are wider than bacillary bodies. The genus contains bacteria causing 3 major diseases of man; tetanus, gas gangrene and food poisoning. Some pathogens, e.g. *Clostridium welchii* now-a-days called *Clostridium perfringens* and *Clostridium tetani* are found normally in human and animal intestine. Clostridia are motile with peritrichate flagella, except *Clostridium perfringens* and *Clostridium type VI*. *Clostridium perfringens* and *Clostridium butyricum* are capsulated while others are not so. Pathogenic clostridia forms powerful exotoxins. *Clostridium botulinum* is non-invasive while *Clostridium tetani* has slight invasive properties. Tetanus results from the action of powerful exotoxin of *Clostridium tetani*.

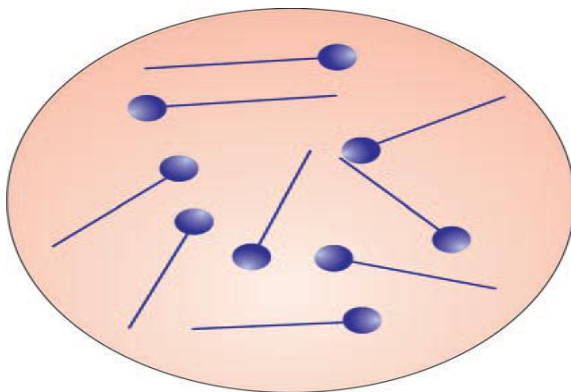
The gas gangrene clostridia are toxigenic and invasive causing even septicemia.

### **CLOSTRIDIUM TETANI**

It is widely distributed in soil and in intestine of man and animals.

#### **Morphology:**

It is slender, long, slightly curved, Gram-positive  $4.8 \mu \times 0.5 \mu$  and occurring singly or in chain. It shows considerable variation in length. Spores are spherical, terminal and bulging, giving the bacilli drumstick appearance. It is non-capsulated and motile.



#### **Cultural characters:**

It is an obligatory anaerobe that grows only in absence of oxygen. The characteristic of anaerobic bacilli is their inability to utilize oxygen as the final hydrogen acceptor. It lacks cytochrome and cytochrome oxidase and is unable to break down hydrogen peroxide because it lacks catalase and peroxidase.

Therefore, hydrogen peroxide tends to accumulate to toxic concentration in the presence of oxygen. It also lacks superoxide dismutase and consequently permit the accumulation of toxic free radical superoxide.

Hence, it can carry out its metabolism only at negative oxidation reduction potential which means an environment that is strongly reducing. The optimum temperature is  $37^{\circ}\text{C}$  and pH 7.4. It grows fairly well in ordinary media. Cultures have burnt organic smell.

**Cooked meat medium:** It grows well in this medium with turbidity and gas formation. The meat is not digested but is turned black after prolonged incubation.

**Nutrient agar medium:** It produces swarming growth forming fine film over the medium. By increasing the concentration of agar in the medium after 2 to 4 days' incubation, colonies are irregularly round, 2 to 5 mm in diameter, translucent, grayish yellow with granular surface and ill-defined edges.

**Blood agar medium:** A zone of  $\alpha$  hemolysis is produced. It later on develops into beta hemolysis, due to production of hemolysin (tetanolysin).

**Lactose egg yolk milk medium:** There is no opalescence, pearly layer, proteolysis or lactose fermentation.

**Biochemical reactions:** It does not ferment any sugar and is slightly proteolytic. It forms indole. Gelatin liquefaction occurs slowly. Coagulated serum is softened. Milk is not coagulated.

**Resistance:** Spores of *Clostridium tetani* withstand boiling for 15 to 90 minutes. Autoclaving at 121°C for 20 minutes kills spores. Spores otherwise can survive in soil for years. Iodine (1% aqueous solution) and H<sub>2</sub>O<sub>2</sub> (10 volumes) kill spores within a few minutes.

There are many clinical types of tetanus:

1. *Tetanus neonatorum*: It occurs from contamination of cut surface of umbilical cord in infants. It has high rate of fatality.
2. *Postabortal and puerperal tetanus*: It results from infection of genital tract with unsterile instrument and dressing. Puerperal tetanus is rare but most dangerous.
3. *Splanchnic tetanus*: There is involvement of muscle of deglutition and respiration with dysphagia.
4. *Cephalic tetanus*: It occurs from the wounds of head. There is unilateral and bilateral contraction of muscles of face.

### Laboratory Diagnosis

The diagnosis is always clinical and bacteriological findings confirm the diagnosis.

1. *Microscopic examination*: Smears from wound material after Gram's staining show Gram-positive bacilli with typical drumstick appearance.
2. *Culture*: Diagnosis by culture is more dependable. Excised bits of tissue from necrotic depth of wound is inoculated into cooked meat broth, blood agar and lactose egg yolk medium. The addition of polymyxin B to which clostridia resist, make the medium more selective.

If the material is grossly contaminated with other organisms, heating at 80°C for 10 minutes may be useful for destroying non-sporing organisms.

**Animal inoculation:** Mouse is a suitable laboratory animal for demonstration of toxigenicity. 2 to 4 days' old cooked meat culture (0.2 ml) is inoculated into the root of tail of a mouse. A second mouse which has received tetanus antitoxin (1000 units) an hour earlier serves as control. Symptoms appear in test animal in 12 to 24 hours with stiffness of tail. Rigidity develops to the inoculated side of the leg, opposite leg, trunk, fore limb in this order. The animal dies within 2 days. However, appearance of ascending tetanus in animal is diagnostic.

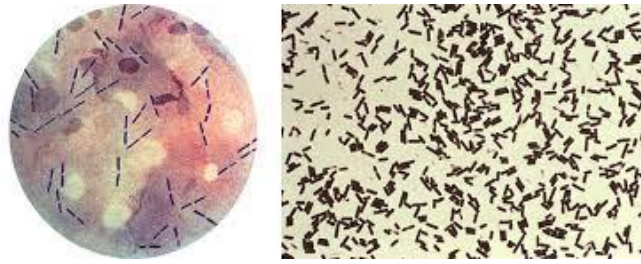


### CLOSTRIDIUM PERFRINGENS (Clostridium welchii)

It is a normal inhabitant of the large intestine of man and animals. It is found in feces and contaminates the skin of perineum, buttocks and thigh. It also produces food poisoning and necrotic enteritis in man.

**Morphology:** It is a plump, Gram-positive bacillus with straight, parallel sides, rounded or truncated ends about 4 to 6  $\mu \times 1 \mu$ . It may occur singly or in chains. It is pleomorphic. Filaments and involution forms are common.

It is capsulated and non-motile. Spores are central or subterminal.



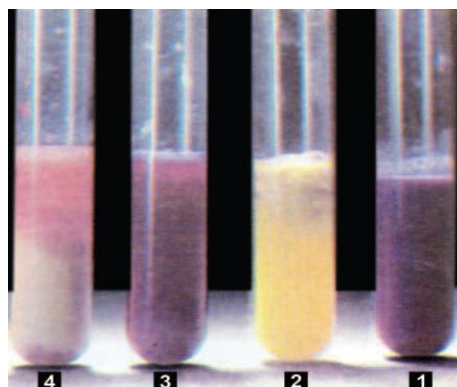
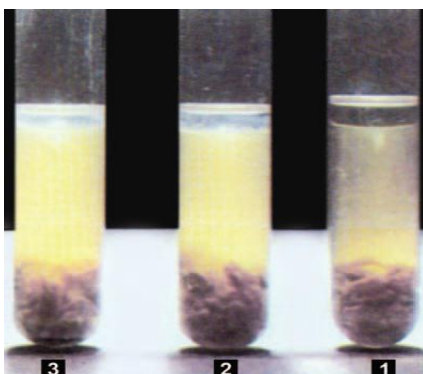
**Cultural characters:** It is an anaerobe, growing rapidly at 37°C.

- a. *Cooked meat medium*: Fairly good growth occurs at 37°C. The medium becomes turbid within 24 hours with production of gas. The meat is turned pink without digestion. The culture has sour odor.
- b. *Nutrient agar*: Two types of colonies appear after 24 hours of incubation; (i) 2 to 4 mm round, smooth, butyrous emulsifiable colonies, (ii) Umbonate colonies with brownish opaque center and lighter radially striated periphery having crenated edges.



**Biochemical reactions:** Glucose, maltose, lactose and sucrose are fermented with production of acid and gas. In litmus milk it produces acid with gas. Milk is disrupted due to vigorous production of gas. This is called stormy clot. Indole is negative and H<sub>2</sub>S is formed abundantly.

**Resistance:** Autoclaving at 121°C for 18 minutes destroys the spores. Spores are resistant to antiseptics and disinfectants in common use.



Cooked meat medium

- 1. Control; 2. *Clostridium sporogenes*;
- 3. *Clostridium perfringens*

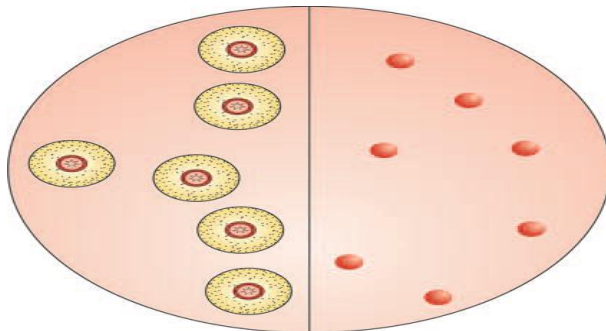
Litmus milk

- 1. Control; 2. *Clostridium perfringens*;
- 3. *Pseudomonas aeruginosa*; 4. *Escherichia coli*

## NAGLER'S REACTION

*Clostridium perfringens* are cultured on plates containing 20 percent of human serum or egg yolk. The organism produces opalescence in media containing human serum and egg yolk. The opalescence is due to lecithinase activity of alpha toxin. Alpha toxin splits lipoproteins and liberates lipids. The lipid deposits around the colony to give opalescence. The reaction is specific and is inhibited by alpha toxin antitoxin sera. It is a useful test for the rapid detection of *Clostridium perfringens* in clinical specimen.

Beta, epsilon and iota toxins have lethal and necrotizing properties. Besides toxins, *Clostridium perfringens* also produces soluble substances with enzymatic properties, e.g. neuraminidase, hemagglutinin, fibrinolysin, hemolysin, histamine, etc.



## Laboratory Diagnosis

### A. Hematological investigation:

- Total leukocyte count usually shows no change. Increased count occurs in secondary infection.
- Differential leukocyte count shows no change.
- Anemia, increased serum, bilirubin and hemoglobinuria may occur due to excessive RBC destruction.

### B. Bacteriological investigation:

*Specimens:* They are collected from:

- Muscles at the edge of affected area.
- Exudate from area where infection appears more active.
- Necrotic tissue and muscle fragment.

## Microscopic Examination

Gram-stained smear shows Gram-positive, long and thick bacilli. Gram-positive bacilli without spore are suggestive of *Clostridium perfringens*.

**Culture:** Material is inoculated on fresh blood agar and cooked meat media. Surface culture is incubated aerobically and an aerobically. Anaerobic culture is studied after 48 to 72 hours of incubation. Further identification is done by:

- Nagler's reaction.
- Biochemical reaction.
- Animal pathogenicity.

Blood collected during bacteremia is cultured in cooked meat medium and glucose broth. It is identified in usual way.

## Animal Pathogenicity

On the hind limb of guinea pig 0.1 ml of 24 hour cooked meat broth is injected intramuscularly.

Death of animal occurs in 24 to 48 hour. Autopsy shows swelling of injected limb with crepitation due to gas formation. The muscle becomes pink. Organism can be recovered from heart and spleen.

## Bacteriological Diagnosis of *Clostridium perfringens* Food Poisoning

From the feces of patient and suspected food, isolation of non-hemolytic, non-motile anaerobic and Gram-positive bacilli is suggestive of *Clostridium perfringens* infection.

## CLOSTRIDIUM BOTULINUM

*Clostridium botulinum* spores are widely distributed in soil, animal manure, sea mud, vegetables, etc. It causes botulism, a severe form of food poisoning.

**Morphology:** It is Gram-positive about  $5\ \mu \times 1\ \mu$ , non-capsulated motile by peritrichate flagella producing subterminal, oval and bulging spores. It shows pleomorphism and occurs either singly or in pairs or chains.

**Culture characters:** It is strict anaerobic. There are 6 different types (A to F). They differ from one another in their culture characters. These types are identified on the base of immunological difference in toxin production. It grows at 20 to 35°C in neutral or slightly alkaline medium.

a. *Cooked meat medium:* After 2 to 4 days' incubation there is abundant growth. There is blackening of meat particles and gas is also produced.

b. *Nutrient agar medium:* Single colony is difficult to get because of tendency to spread. Colonies develop after 48 hours.

Colony is irregular, 3 to 8 mm, glistening and with granular surface. Consistency of colony is butyrous and is emulsified easily.

c. *Blood agar:* Colonies on blood agar medium are hemolytic.

d. *Lactose, egg yolk, milk agar medium:* All types of this organism produce opalescence and pearly layer. All are lactose negative.

**Resistance:** Spore is highly resistant, surviving several hours at 100°C. It withstands 120°C for 20 minutes. However, heat resistance is diminished at acid pH or high salt concentration.

**Biochemical reactions:** All types ferment glucose, maltose with acid and gas. There are two biochemical types of *Clostridium botulinum*:

a. Proteolytic (types A, B and F).

b. Saccharolytic and non-proteolytic (type C, D and E). H<sub>2</sub>S is produced by all types.

## Laboratory Diagnosis

### Bacteriological Investigations

**Specimen:** Diagnosis is based on demonstration of bacillus or toxin in food or feces. In early stages toxin may be detected from patient's blood.

**Culture:** Isolation of organism (toxigenic strain) from vomit, food or feces in absence of toxin is of no significance.

### Demonstration of *Clostridium botulinum*

**toxin:** Specimen like food, vomit, etc. are ground up and soaked overnight in equal volume of isotonic saline solution. It is centrifuged, and supernatant is divided into 3 parts. One portion is heated at 100°C for 10 minutes. Penicillin is added (concentration being 100 units/ml).

One of the guinea pig is protected with polyvalent botulinum antitoxin and 2 ml of unheated material is injected intraperitoneally only. Unheated material (2 ml) is injected into second guinea pig. The third animal is injected with 3 ml of heated material. Second guinea pig develops toxin symptoms like dyspnea, flaccid paralysis and dies within 24 hours. First and third animals show no toxic symptoms.

Typing is done by passive protection with type specific antitoxin. A retrospective diagnosis may be made by detecting of antitoxin in patients serum.

