

Practical No. 15

Genus Clostridium

Clostridium is G+ve, obligate anaerobic spore-forming bacilli.. Four main species responsible for disease in humans:

Clostridium perfringens;

- ★ Gram +ve, non-motile, obligate anaerobe, spore-forming, capsulated in tissues or young cultures, rounded end bacilli.
- ★ Spores are oval, central or subterminal, not bulging.
- ★ The strains that produce more spores produce less toxins, and vice versa.
- ★ Grow best on media containing carbohydrates such as; glucose agar, or glucose blood agar.
- ★ Spores of type A (of types A-F) resist boiling for few minutes, while strains of food poisoning resist boiling for 1-3 hours. Other strains can resist boiling for several hours.
- ★ Produce narrow zone of complete hemolysis (theta toxin), while α - toxin produce a wide zone of hemolysis.
- ★ Direct smear from deeper parts of the wound may show 3 types of Clostridia;
 - 1- *C. perfringens*; thick Gram +ve bacilli
 - 2- *C. septicum*; citron bodies, boat-shape, pleomorphic, with irregular staining.
 - 3- *C. novyi*; Large bacilli, with oval subterminal spores.

Clostridium tetani;

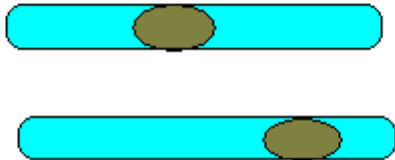
- ⊕ Straight, slender, rod-shaped, rounded ends, curved or filamentous.
- ⊕ Motile with peritrichous flagella
- ⊕ Spores are rounded or oval terminal, form drum-stick appearance.
- ⊕ Spores are highly resistant, may remain alive for years, and resist boiling for 15-90 min., some may resist dry heat at 150°C for 1 hour.

Clostridium botulinum;

- ★ Rod-shaped, single or in pairs or in chains
- ★ Spores are oval, subterminal, bulging
- ★ Spores formed best in sugar-free medium.
- ★ Motile with 4-8 peritrichous flagella
- ★ Form large irregular, glistening colonies with fimbriated border.
- ★ Meat is digested in cooked meat medium with putrid odour.
- ★ Spores are destroyed at 180°C, and in dry heat for 5-15 min., and in boiling for 5 hours.



C tetani



C perfringens



C botulinum

The fourth species is *C.difficile* which overgrows during antibiotic therapy, also may lead to *pseudomembrane colitis*.

Clostridium spp. could be divided into;

1. Saccharolytic organisms; *C. perfringens*, ferments carbohydrates, produces acid and gas
2. Proteolytic organisms; *C. tetani*, digest proteins with blackening and bad smell production.
3. Mixed saccharolytic & proteolytic; certain groups of *C. botulinum*.

Laboratory diagnostic tests;

Specimen; Wound, food, stool.

1- Gram stain;

2- Culture; Cultures are incubated under anaerobic conditions.

I- Blood agar, most clostridia produce flat, gray, spreading colonies somewhat resembling those of *Bacillus* sp. They are also usually hemolytic. *Clostridium perfringens* produces raised, entire colonies with double zone hemolysis, the complete zone round the colony is due to theta toxin, and the wider zone around it is due to alpha toxin.

II- Robertson's medium (cooked meat medium); Cooked Meat Medium provides a favorable environment for the growth of anaerobes, since the muscle protein in the heart tissue granules is a source of amino acids and other nutrients. The muscle tissue also provides reducing substances, particularly glutathione, which permits the growth of strict anaerobes. The sulfhydryl groups, which exert the reducing effect, are more available in denatured protein; therefore, the meat particles are cooked for use in the medium. Cooked Meat Medium is still widely used for the cultivation and maintenance of clostridia and for determining proteolytic activity of anaerobes.

On Robertson's cooked meat medium → blackening of meat will be observed with the production of H₂S and NH₃ (in the case of *C. tetani*).

III- Brewer Thioglycollate Medium ; contains highly nutritious proteose peptone and beef infusion which support the growth of fastidious bacteria. Sodium thioglycollate helps to create anaerobic conditions. Methylene blue indicates oxygen content of the medium by exhibiting a bluish-green color of the medium in the presence of oxygen. Brewer's anaerobic jar is used for growing bacteria under anaerobic condition.

3- Biochemical tests; stormy fermentation

In anaerobically-grown Litmus Milk cultures, enzymes of *C. perfringens* will attack the proteins and carbohydrates of the milk producing a "stormy fermentation" with acid production (the indicator, litmus turns pink), clotting of milk proteins, and gas formation.

4- Serology test; Nagler's reaction for the detection of lecithinase activity;

A test for the identification of alpha toxin of *Clostridium perfringens*; the addition of antitoxin to cultures on egg yolk agar prevents visible opacity, due to lecithinase action which is normally observed around colonies.

An egg-yolk plate is divided into two halves. Over one half is spread with a specific antitoxin. The culture to be tested, together with a positive control is streaked across the plate, going from the untreated area of the plate to the area that is covered with antitoxin. The culture is then incubated anaerobically. Following incubation, lecithinase activity, caused by the action of the *Clostridium*

perfringens a-toxin is seen as a precipitate in the medium on the side of the plate that did not receive the antitoxin, but not on the side that was treated.