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Clostridium spp.

Clostridium is a genus of Gram-positive bacteria, which includes several significant human pathogens.

There are four medically important species: ***Clostridium tetani***, ***Clostridium botulinum***, ***Clostridium perfringens***, and ***Clostridium difficile***. All clostridia are anaerobic, spore-forming, gram positive rods.

1. *Clostridium tetani*

- *C. tetani* causes tetanus.

- *C. tetani* is a rod-shaped Gram-positive bacterium. It is motile by way of various flagella that surround its body. *C. tetani* is an anaerobe and cannot survive in the presence of oxygen. It is non-capsulated. Each cell can form a single spore, generally at one end of the cell, giving the cell a distinctive drumstick shape or tennis rackets.

- Spores are widespread in soil. The portal of entry is usually a wound site.

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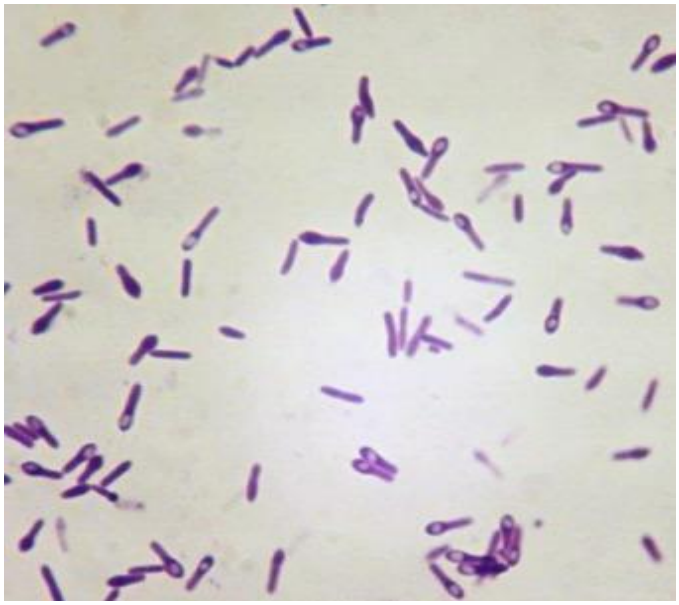


- **Diagnosis**

Media used:

1- Robertson's Cooked Meat (RCM)

Robertson's Cooked Meat (RCM) medium is used for the cultivation of aerobic, microaerophilic, and anaerobic microorganisms, especially *Clostridium* species. It is also known as Cooked Meat Broth (CMB) as it contains pieces of fat free minced cooked meat of ox heart and nutrient broth.

A saccharolytic reaction is shown by change color of the meat to pink with a rancid smell due to carbohydrate decomposition. **A proteolytic reaction** is shown by blacking of the meat with a very unpleasant smells due to protein decomposition.

1. *Clostridium perfringens*: Saccharolytic anaerobes (turn the color of meat pieces into pink)
2. *Clostridium tetani:* Proteolytic anaerobes (blacking of the meat)

2- **Blood agar** the bacilli produce a swarming (thin spreading film) growth. The colonies of *Cl. tetani* are surrounded by a zone of α-hemolysis, which subsequently develops into β-hemolysis, due to the production of an oxygen-labile hemolysin known as tetanolysin.

On Blood Agar
C. tetani produces
a fine film of growth.
Use a hand lens to
examine the plate.

On fresh blood agar
C. tetani is haemolytic
(alpha first followed by
beta haemolysis).

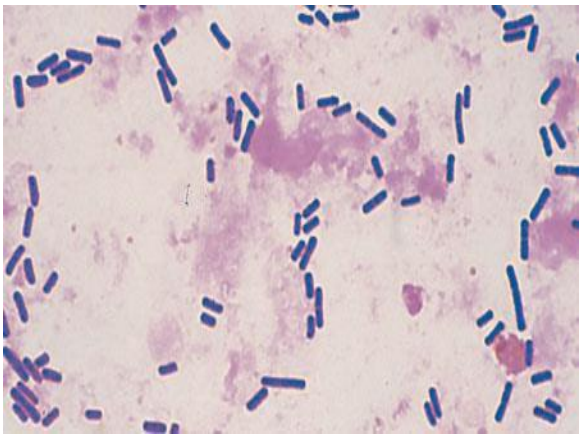
It is indole positive and MR, VP, H2S and nitrate reduction negative.

2- Clostridium perfringens

large **gram-positive** bacillus with straight, parallel sides and rounded or truncated ends, occurring singly or in chains or small bundles. It is capsulated and non-motile. Spores are central or subterminal but are rarely seen.

- Skin and Soft tissue: gas gangrene, cellulitis

- Gastrointestinal: necrotising enteritis, food poisoning



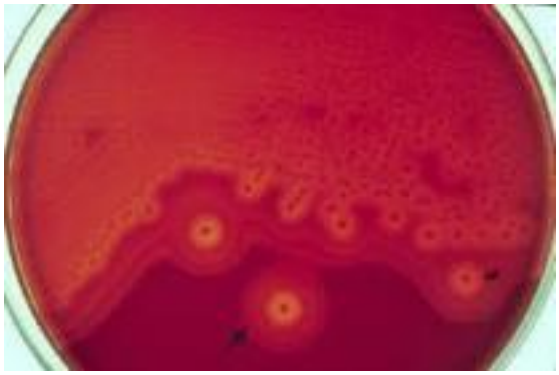
Diagnosis

It is an **anaerobe** but can also grow under **microaerophilic** conditions. It grows over a pH range of 5.5 to 8.0 and temperature range of 20°C to 50°C (optimum temperature range 37-45°C).

Media used:

1-On Robertson's cooked meat medium in which meat is turned pink and not digested.

2-On blood agar: It forms spreading colonies on blood agar surrounded by a double zone of hemolysis known as target hemolysis (i.e. inner narrow zone of complete lysis due to θ-toxin (theta) and wider outer zone of partial hemolysis due to α-toxin).



Biochemical test

- It is **actively saccharolytic**. Glucose, maltose, lactose and sucrose are fermented with the production of **acid** and **gas**.
- It is **indole** negative, **MR** positive and **VP** negative.

3- Clostridium botulinum

C. botulinum is a strictly anaerobic gram-positive bacillus. It is non-capsulated, motile with peritrichous flagella and produces spores which are oval, sub-terminal and bulging.

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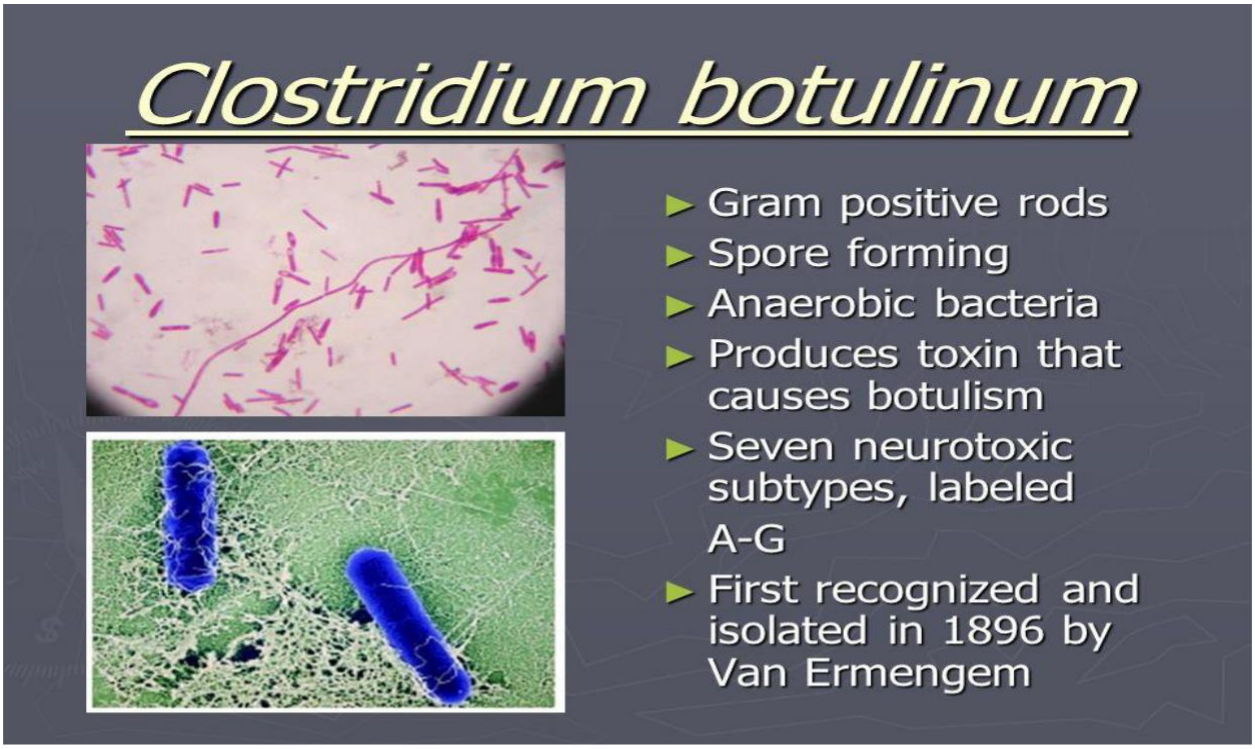

On Robertson's cooked meat medium *C. botulinum* is proteolytic.

4-*Clostridium difficile*

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Basic Characteristics	Properties (*Clostridium difficile*)
Capsule	Capsulated
Catalase	Negative (-ve)
Flagella	Flagellated
Gram Staining	Positive (+ve)
H2S	Positive (+ve)
Hemolysis	Negative (-ve)
Indole	Negative (-ve)
Motility	Motile
Oxidase	Negative (-ve)
Shape	Rods
Spore	Positive (+ve)
VP (Voges Proskauer)	Negative (-ve)