CLOSTRIDIUM SPECIES

- The clostridia are **large anaerobic** (a few species are aerotolerant), gram-positive, motile rods. Many decompose proteins or form toxins, and some do both.
- Their natural habitat is the soil, marine sediments, sewage, or the intestinal tract of animals and humans, where they live as saprophytes.
- **Spores** of clostridia are usually wider than the diameter of the rods in which they are formed.
- Some clostridia produce **large raised** colonies (eg, *C perfringens*); others produce **smaller** colonies (eg, *C tetani*). Some clostridia form colonies that spread or **swarm** on the agar surface (*Clostridium septicum*). Many clostridia produce a zone of β-hemolysis on blood agar. *C perfringens* characteristically produces a **double zone of β-hemolysis** around colonies.
- The important pathogenic species are:
  - *Clostridium botulinum*: Causes botulism
  - *Clostridium tetani*: Causes tetanus
  - *Clostridium perfringens*: Causes gas gangrene and food poisoning
  - *Clostridium difficile*; causes pseudomembranous colitis and antibiotic-associated diarrhoea.
- Note: the toxins produced by the organisms of tetanus and botulism attack nervous pathways (**neurotoxins**), the organisms associated with gas gangrene attack soft tissues by producing toxins (**histotoxic**). *C difficile* and some strains of *c. perfringens* produce an **enterotoxin**
- *C botulinum* produces botulinum toxin, one of the most potent neurotoxins on the planet, responsible for botulism, a disease characterized by **flaccid paralysis**.
- *C tetani* also produces a neurotoxin, tetanospasmin, that blocks release of inhibitory neurotransmitters resulting in tetanus, a disease characterized by **spastic paralysis**.
- tetanus is a cause of death in newborn infants when the umbilical stump becomes infected due to the cord being cut with a contaminated blade.

C. perfringens is a facultative anaerobe which can be cultured, both anaerobically and microaerophilically. Optimum temperature range is 37–45 ºC.

Typical food poisoning strains produce heat resistant spores that can survive boiling for several hours, whereas the spores of strains that cause gas gangrene are inactivated within a few minutes by boiling.

Gas Gangrene: characterized by rapidly spreading oedema, myositis, necrosis of tissues, gas production and profound toxaemia occurring as complication of wound infection.

The main source of organism is animal and human excreta, and spores are distributed widely (soil and even air). The skin often bears spores, especially, in areas of the body that may be contaminated with intestinal organisms.

Pathogenesis:

Impairment of the normal blood supply of tissues with a consequent reduction in oxygen tension may allow an anaerobic focus to develop.

The organisms multiply rapidly and produce a range of toxins (these will damage tissue). Then, they spread into adjacent viable tissue, particularly, muscle, kill it and render it anaerobic and vulnerable to further colonization with the production of more toxin and aggressins.

Hyaluronidase produced by c. perfringens breaks down intercellular cement substance and promotes the spread of the infection along tissue.

Collagenase (liquefy muscle)

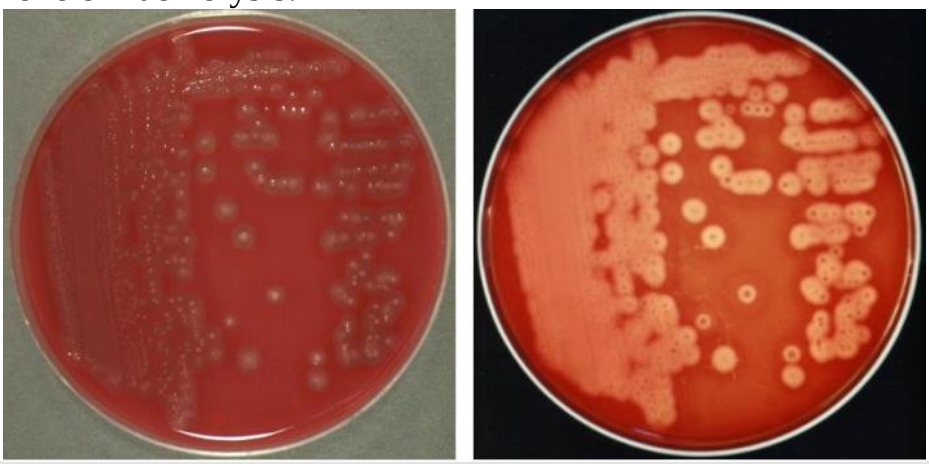
Toxins: Alpha toxin, a phospholipase C (lecithinase). Theta toxin: It has hemolytic and necrotic effect on tissue.

Lab finding

Specimen: pus, necrotic tissue, feces, food

Smears: large gram positive rods with or without spores

Blood agar: Large *beta*-haemolytic colonies are produced (most food-poisoning strains are non-haemolytic). Some strains produce a double zone of haemolysis.



The same Blood Agar plate examined with transmitted light. Colonies are surrounded by a double-zone haemolysis, which consists of an inner clear zone and an outer hazy zone.

Stormy fermentation:
A descriptive term referring to the appearance of the turbid reaction in litmus milk with coagulation andgas production, in which milk is converted into a coagulum with entrapped bubbles of gas

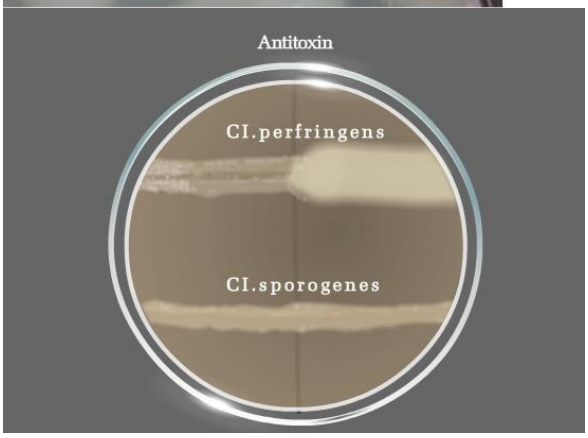
Neomycin blood agar
This selective medium is recommended for the isolation of *C. perfringens* from sites likely to contain several organisms, e.g. from wounds. When incubated anaerobically, the growth of facultative Gram negative rods is inhibited.
Robertson's cooked meat medium (RCMM): In this medium *C. perfringens* is saccharolytic and slightly proteolytic. Gas is formed.

Saccharolytic and proteolytic reactions in RCMM
A saccharolytic reaction is shown by reddening of the meat with a rancid smell due to carbohydrate decomposition.
A proteolytic reaction is shown by blackening of the meat with a very unpleasant smell due to protein decomposition.

Clostridia are catalase and oxidase negative. The most useful biochemical reactions which help to identify *C. perfringens* and other pathogenic *Clostridium* species is culturing the organism anaerobically on lactose egg yolk medium. This medium tests for lecithinase C activity, lipase hydrolysis, lactose fermentation and proteinase activity.

Lecithinase C activity: Seen as an opacity in the medium due to the breakdown of lecithin in the egg yolk.
Lipase hydrolysis: Seen as a pearly (fatty) layer covering colonies and sometimes extending into the medium A restricted intense area of opacity is also produced in the medium.
Lactose fermentation: There is a reddening in the medium. The colonies become red on exposure to air.
Proteinase activity (proteolysis): Shown by an area of clearing around the colonies due to the breakdown of casein in the milk by the enzyme proteinase.

On lactose egg yolk medium, *C. perfringens*:
● Produces lecithinase C (*alpha* toxin)
● Ferments lactose
● Does not hydrolyze lipid
● Shows no proteinase activity
C. perfringens
produces an opacity in medium containing lecithin due to lecithinase C activity (*alpha* toxin). This opacity can be inhibited by applying specific antitoxic serum to the medium which will inactivate the lecithinase. The technique is referred to as the Nagler reaction.



Method of performing Nagler test
1. Prepare a plate of lactose egg yolk milk agar

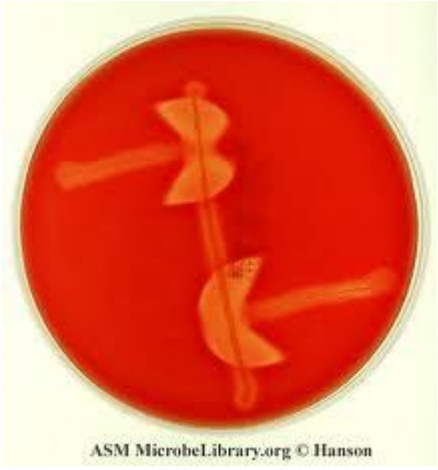
*The Nagler reaction can also be demonstrated using serum agar or egg yolk agar, but lactose egg yolk milk medium is preferred because it differentiates *C. perfringens* (lactose fermenting) from other clostridia that give a positive Nagler reaction.
2. Turn the plate over, and using a wax pencil, draw a line across the centre of the plate.
3. Using a sterile swab, cover one half of the medium with *C. perfringens* antitoxin. Allow to dry.
4. Inoculate the test organism at right angles to the centre line so that the inoculum passes from the antitoxin-free half of the plate to the antitoxin-covered half. Inoculate also a non-toxin producing control organism that will grow anaerobically.
5. Incubate the plate anaerobically at 35–37 ºC overnight.
6. Look for an opacity around the inoculum in the half of the plate containing no antitoxin and no opacity in the half containing the antitoxin.

Reverse CAMP test

Because of the difficulty in obtaining *C. perfringens* antitoxin to use in the Nagler reaction, some laboratories use a reverse CAMP technique to assist in the identification of *C. perfringens*.

Reverse CAMP test

It can be used for differentiation of *Clostridium perfringens* from other Clostridium species. Here, a CAMP positive Group B Streptococcus is streaked in the center of sheep blood agar, and *Clostridium perfringens* is streaked perpendicular to it. Following incubation at 37oC for 24-48 hours in anaerobic conditions, an "arrowhead" hemolysis is seen between the growth of *Clostridium perfringens* and Group B Streptococcus. This is because of alpha toxin produced by *Clostridium perfringens* interacts with CAMP factor and produce synergistic hemolysis.



C. botulinum
Strict anaerobe. Grows best at 30–35 ºC.
Types of Clostridium botulinum: They are from A – H according to the type of toxin produced.

- Types A, B, and E are the most commonly associated with illness and have the following characteristics:

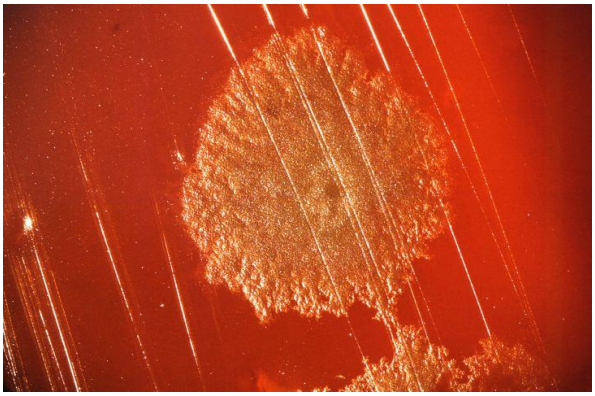
1. They are among the most **highly toxic** substances known.
2. They are **neurotoxic proteins** (MW = 150 000)
3. Lethal dose for human is low
4. They are **destroyed by heating** for 20 minutes at 100 C
5. Toxin **production is under the control of a viral gene** (Bacteriophage yielded from toxigenic strain and it may infect non-toxigenic strain and convert it to toxigenic).

Action of botulism toxin
It is a neurotoxic protein. All of its types (A, B, and E) are made of **heavy and light** chains linked by disulfide bonds. The heavy chain is thought to bind the toxin to the motor nerve end plate. The light chain blocks the calcium-mediated release of acetyl choline. The toxin acts by blocking the release of acetyl choline at synapses and neuromuscular junctions causing flaccid paralysis

Pathogenesis
Botulism is intoxication. It results from ingestion of food in which Clostridium botulinum spores germinate and produce toxins under anaerobic conditions. These foods are spiced, smoked, vacuum-packed, or canned alkaline foods. The toxin acts by blocking the release of acetyl choline at synapses and neuromuscular junctions causing flaccid paralysis.
Infant botulism …
It may result from honey feeding and cause signs of paralysis or sudden death.

Robertson's cooked meat medium (RCMM): Inoculate the emulsified specimen (in 0.1% peptone water) in several containers of RCMM. Heat half of them at 80 ºC for 30 minutes (spores remain). Incubate the heat treated and untreated inoculated RCMM at 35 ºC for 3–5 days. Types A, B and F blacken and digest cooked meat medium (proteolytic reaction) and produce hydrogen sulphid gas (types C, D, and E do not).

Blood agar subculture from RCMM (anaerobic culture): *C. botulinum* produces large semi-transparent colonies with a wavy outline. Most strains are *beta*-haemolytic.



Lactose egg yolk milk agar
C. botulinum hydrolyzes lipid (pearly opalescence). Types A, B, and F show proteinase activity (area of clearing around the colonies). Lactose is not fermented.

C. tetani
Strict anaerobe with a temperature range of 14–43 ºC (37 ºC optimum).
General characteristics:
• Worldwide in distribution in the soil and in animal feces
• Longer and thinner gram-positive rods with round terminal spores giving characteristic "**drum-stick**" appearance.
• There are ten antigenic types of c. tetani but all produce the same neurotoxin.
• The toxin has two components:
1. Tetanospasmin: Neurotoxic property
2. Tetanolysin: Hemolytic property

Pathogenesis:
Clostridium tetani is not an invasive organism. The infection remains strictly localized in the area of dead tissue (into which the spores have been introduced). Germination of spores to vegetative organisms that produce toxin Tetanospasmin will reach the CNS via the blood and result in generalized muscular spasm.
…Clinical findings of tetanus …

Duration is 4 to 5 days – many weeks. There is muscular contraction of the voluntary muscles(1st area of infection) then the muscles of the jaw (Lock-Jaw disease). Later, other voluntary muscles are involved causing generalized spasm resulting in respiratory paralysis and cardiac failure which lead to death (50%).
 Tetanus neonatrum: Follows contamination of the umbilical cord of newborns when it is cut by contaminated food.

Blood agar: When isolated (only very occasionally), *C. tetani* produces a fine film of feathery growth. Use a hand lens to examine the plate.
On fresh blood agar, *C. tetani* is haemolytic (*alpha* first followed by *beta* haemolysis).

Antitoxin test

If growth occurs, subculture on a blood agar antitoxin plate (half the plate covered with antitoxin). Incubate the plate anaerobically. The haemolysis produced by *C. tetani* is inhibited by the antitoxin.

Robertson's cooked meat medium (RCMM): *C. tetani* is slowly proteolytic.

Prevention …
Active immunization with toxoid (detoxified toxin) to stimulate Ab.
Proper care of wound (Remove the necrotic tissue)
Prophylactic use of antitoxin.
Administration of penicillin (to inhibit Clostridium and pyogenic bacteria)
 Treatment with antitoxin in tetanus neonatrum is life saving.
…Control …
Active immunization of children with tetanus toxoid 3 injections:
 In the 1st year
 Booster injection at entry to school
 Boosters are spaced 7-10 years
Usually in young children: In immunization, tetanus toxoid is combined with diphtheria toxoid and Pertussis vaccine (DTP

C. difficile
Motile, g +ve, oval subterminal spores.
It occurs commonly in faeces of neonates and babies.
It produces an enterotoxin (toxin A) and a cytotoxin (toxin B).
It is a proven cause of antibiotic associated diarrhea, leading to life-threatening condition, pseudomembranous colitis. There is history of antibiotic use (clindamycin, cephalosporins)

Diagnosis:
Sample-faeces (enrichment and selective procedure)
Toxin detection in feces (ELISA)