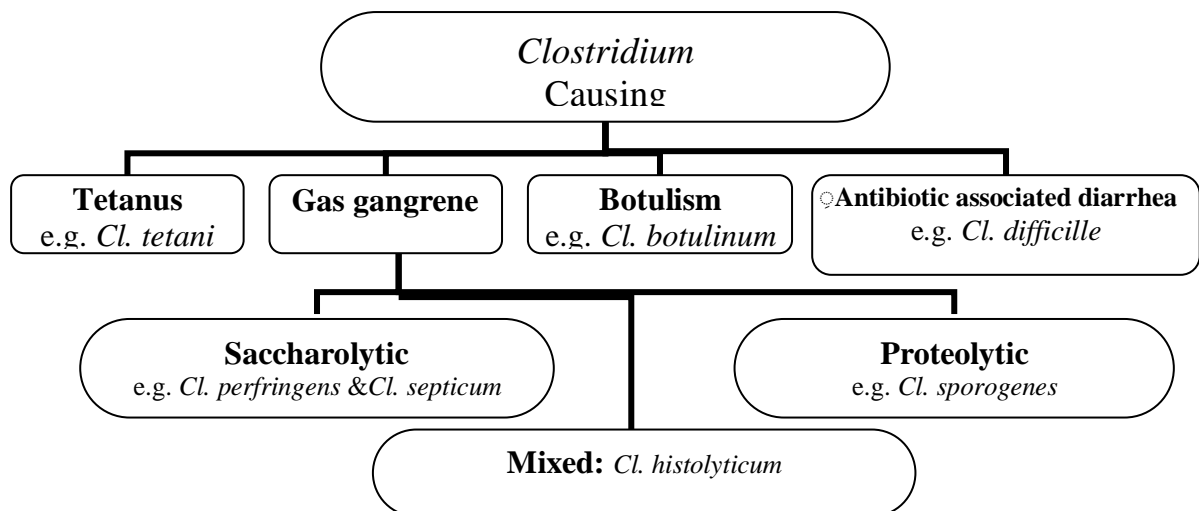
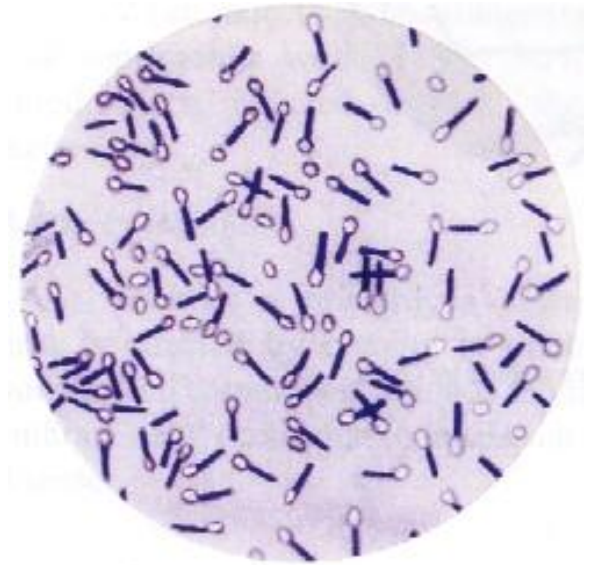


Practical NO 5

Clostridium spp

Clostridia are:

- Large Gram positive
- Straight or slightly curved rods with slightly rounded ends
- Anaerobic bacilli
- Spore forming
- Saprophytes
- Some are commensals of the animal & human gut which invade the blood and tissue when host die and initiate the decomposition of the corpse (dead body)
- Causes diseases such as gas gangrene, tetanus, botulism & pseudo-membranous colitis by producing toxins which attack the neurons pathways



Anaerobic Cultivation

Removal of oxygen & replacing it with inert gas

Anaerobic Jar



Culture Media containing reducing agent

Thioglycollate broth

Sodium thioglycollate (Reducing agent)

Cooked Meat Medium

Meat particles (prepared from heart muscles) which contain hematin & glutathione that act as reducing agent

***Clostridium*-causing tetanus (*Clostridium tetani*)**

a) Description

- Gram positive, straight, slender rod with rounded ends
- Obligate anaerobe
- Motile and has numerous peritrichous flagella
- Grows well in cooked meat broth and enriched blood agar
- Spores are highly resistant to adverse conditions
- Spores of some strains resist boiling in water for up to 3h. They may resist dry heat at 160 °C for 1 h and 5% phenol for 2 weeks or more
- Iodine (1%) in water is able to kill the spores within a few hours



b) Occurrence of tetanus bacilli

- Occurs in the intestine of humans and animals
- Derived primarily from animal faeces and indirectly via soil
- Especially prevalent in manure soil

c) Tetanus and clinical symptoms

- *Cl. tetani* is the causative agent of tetanus
- In developing countries most cases occur in
 - Children secondary to wounds
 - Neonates with contaminated umbilical stump after labor or abortion
 - After surgical procedures done with non-sterilized instruments
 - Also due to use of badly sterilized cut gut "surgical ligatures"
- The spores vegetate and the organism multiply locally at the site of infection producing exotoxins (neurotoxins)
- Toxins spreads via
 - The toxins is absorbed from the site of its production in an infective focus and is transmitted to the CNS via motor nerves and apparently specifically by motor fibers.

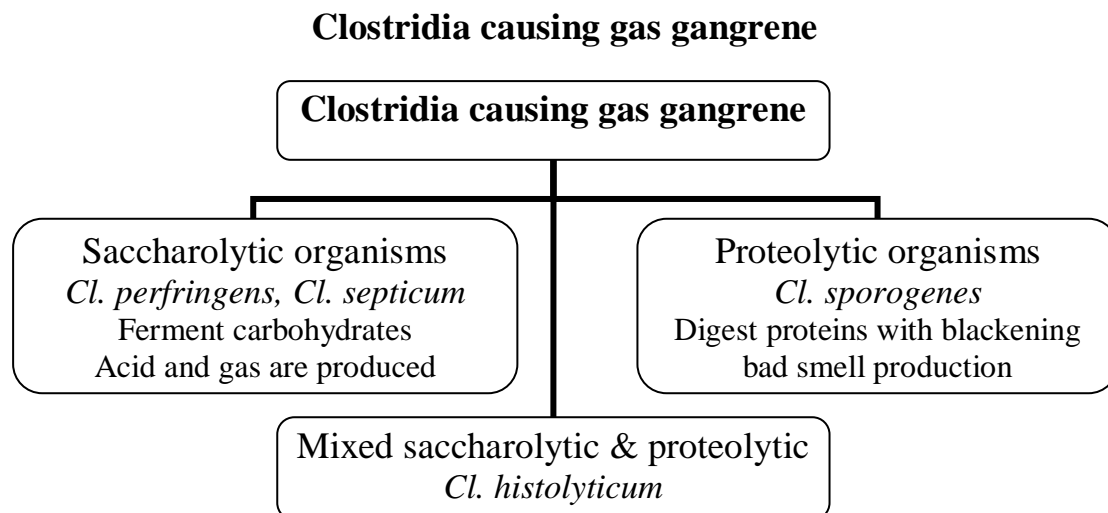
- Via blood to all nerves & the subsequent transmission to the CNS
- The period between injury and the first signs is usually about 10-14 days
- Onset of signs and symptoms of tetanus is gradual, usually starting with some stiffness and perhaps pain or near a recent wound
- The toxin act by interfering with synaptic transmission and leads to increased excitability of motor nerve cells and produces tonic spasms.
- The spasm of master muscles (Trismus or Lock Jaw) develops early.
- When tetanus occurs naturally, the tetanus bacilli stay at the site of the initial infection and are not generally invasive, but the toxin diffuses to affect the relevant level of the spinal cord (**local tetanus**) and then to affect the entire system (**generalized tetanus**).

d) Toxin

- *Cl. tetani* produces two types of toxins
 - **Tetanolysin**, which causes lysis of RBCs
 - **Tetanospasmin** is neurotoxin and essential pathogenic product

e) Laboratory Diagnosis

- The diagnosis of tetanus depends primarily upon the clinical manifestation of tetanus including muscle spasm and rigidity.
- **Specimen** from wound exudates using capillary tube
- **Culture:** On blood agar and incubated anaerobically. Growth on blood agar often appears as a fine spreading film.
- **Gram stain** is a good method for identifying *Clostridium*. *Cl. tetani* is Gram positive rod motile with a round terminal spore giving a drumstick appearance.

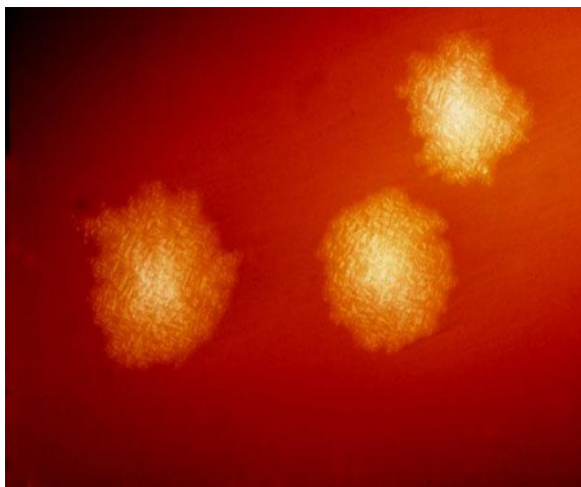
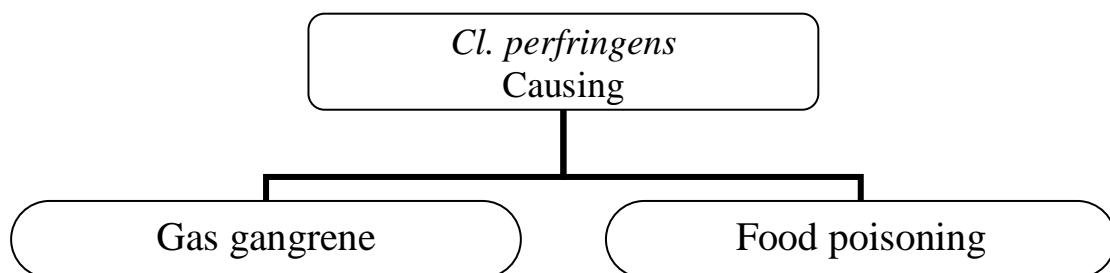


- All of these organisms are **gram positive rods**, their most characteristics feature is the **formation of spore**, which is produced under drastic conditions
- **Gas gangrene is only likely to occur when:**
 - There is extensive damage to tissues
 - Interruption of blood supply

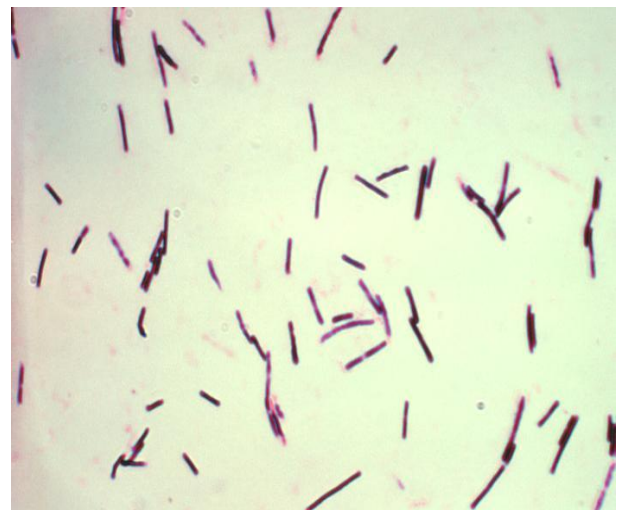
- Contamination of wound with soil and other foreign material
 - Such conditions are particularly associated with
 - War wounds
 - Accidental fracture
 - Industrial injuries
 - Uterine infections may occur after septic abortion
- **The main source of the organisms is animal and human excreta**

Saccharolytic organisms causing Gas gangrene

I- *Clostridium perfringens*



Cl. Perfringens colonies on blood agar after 24 hrs



Gram stain of *Cl. perfringens*

a) Description

- Large Gram-positive bacilli with stubby ends
- **Capsulated**
- **Non motile**
- Anaerobic
- Grown quickly on selective media

- Can be identified by **Nagler reaction**

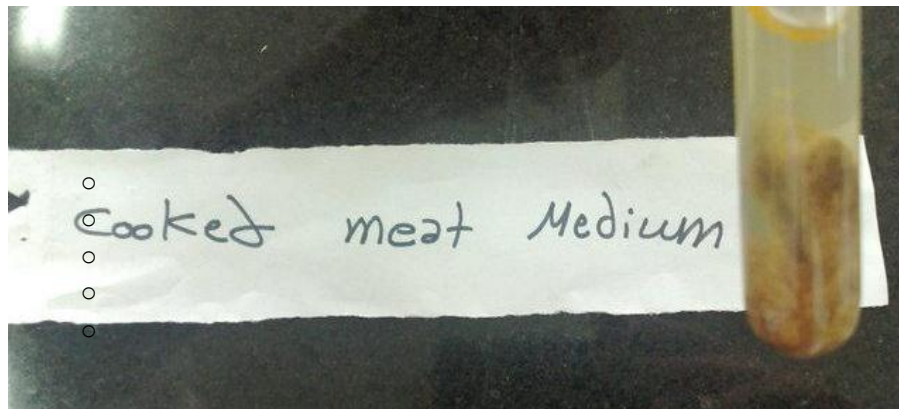
b) Pathogenesis of gas gangrene

- Gas gangrene is life threatening infection of skeletal muscles
- The organism is a normal constituent of the fecal flora
- Impairment of the normal blood supply of tissue with a consequent reduction in oxygen tension may allow an anaerobic focus to develop
- When clostridial infection has been initiated in a focus of devitalized anaerobic tissue, the organisms multiply and produce a range of toxins.
- They spread and attack adjacent viable tissue, particularly muscle fibers, kill it & render it anaerobic, causing fermentation of muscle glycogen with production of acid and gas
- The gas separates the muscle fibers from its sheath, cutting off blood supply leading to necrosis.
- The organism is further colonized with the production of more toxins.
- The incubation period is 1-4 days and earliest symptom is severe pain at the site of wounds.
- In puerperal infections or in cases of septic abortion, the organisms may gain access from faeces-contaminated perineal skin or contaminated instruments to necrotic or devitalized tissues in the uterus. Here they set up a dangerous & often fulminating pelvic infection, possibly with prompt invasion of the bloodstream
- *Cl. perfringens* may also participate in peritoneal infections occurring as a result of extension of pathogens from the alimentary or intestinal obstruction or mesenteric thrombosis
- **The toxins of *Cl. perfringens* :**
 - **α toxin** is the most important toxin
 - Lyses of RBCs, platelets, leucocytes and endothelial cells
 - Increased vascular permeability with massive hemolysis and bleeding tissue destruction
 - Hepatic toxicity and myocardial dysfunction
 - **β-toxin** is responsible for necrotic lesions in necrotizing enterocolitis
 - **Enterotoxin** is heat labile toxin produced in colon → food poisoning

c) Laboratory Diagnosis

- **Specimen:** Histological specimen or wound exudates
 - Histological specimen transferred aseptically into a sterile screw-capped bottle & used immediately for microscopical examination & culture
 - Specimens of exudates should be taken from the deeper areas of the wound where the infection seems to be most pronounced
- **Gram stain:**
 - Gram-positive bacilli, **non motile**, **capsulated** & sporulated. The spore is oval, sub-terminal & non bulging. Spores are rarely observed.
- **Culture:** anaerobically at 37°C

- **On cooked meat medium** → blackening of meat will be observed with the production of H_2S and NH_3



Meat broth was injected with *Clostridium perfringens*. Gas production by *Clostridium perfringens* following injection of the contaminated anabolic steroids caused meat to float to the top of the vial (right).



Reaction on Cooked Meat Medium

Saccharolytic reaction

It causes fermentation of glycogen of muscles

Production of acid and gas

Meat particles remain intact

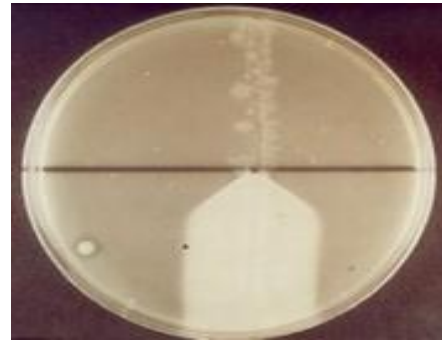
e.g *Cl. perfringens*

Proteolytic Reaction

It causes digestion of meat particles

Formation of black, foul smelling due to sulfur compounds

- **On blood agar** → β -hemolytic colonies
- **Biochemical reactions**
 - *Cl. perfringens* ferment many carbohydrates with acid and gas.
 - *Cl. perfringens* acidified litmus milk with stormy clot production
 - **Nagler's reaction:**
 - This test is done to detect the lecithinase activity
 - The microorganism is inoculated on the medium containing human serum or egg yolk (contains lecithin), colonies of *Cl. perfringens* are surrounded by zones of turbidity due to lecithinase activity and the effect is specifically inhibited if *Cl. perfringens* antiserum containing α antitoxin is present on the medium



The Nagler test for the production of α -toxin by *Clostridium perfringens*

***Cl. perfringens* food poisoning**

- *Cl. perfringens* is normally present in considerable numbers in human faeces
- These bacteria also occur in animals; thus, meat is often contaminated with their heat-resistant spores
- When meat is cooked in bulk, heat penetration is slow and subsequent cooling is slow unless special precautions are taken. The heat-resistant spores may survive and during the cooling period, they will germinate in the anaerobic environment produced by the cooked meat and multiply. Thus, anyone who eats this will consume the equivalent of a cooked meat broth culture of the organism. The organisms are protected from the gastric acid by the protein in the meal and pass in large numbers into the intestine where they sporulate
- **Enterotoxin** released in the gut will cause abdominal cramps about 8-12 h after ingestion, followed by diarrhea. Fever and vomiting are not typically encountered and symptoms generally subside within a day or two.
- Symptoms are similar to food intoxication with *B. cereus* "Diarrheal form"

Laboratory Diagnosis

- This depends upon the isolation of *Cl. perfringens* from the faeces of patients & from those at risk who have eaten the suspected food, and from the food itself.

***Clostridium*-causing botulism (*Clostridium botulinum*)**

a) Pathogenicity

- Botulism (food poisoning)

b) Rout of infection

- Ingestion of food containing botulinal toxin, often from eating of canned food

c) Description

- Strict anaerobic Gram positive bacillus
- Motile with peritrichous flagella
- Spores are oval and sub-terminal
- It is widely distributed saprophyte occurring in soil, vegetables, fruits, leaves
- Produce a potent neurotoxin in food

d) Toxins of *Cl. botulinum*

- Botulinal toxins are among the most poisonous natural substances known
- During the growth of the microorganism, toxin is liberated into the food
- Classified into seven antigenic types (A-G) with types A, B and E most frequently associated with human disease

f) Clinical Features

- The period between ingestion of the toxin and the appearance of signs and symptoms is usually **1-2 days**, but it may be much longer
- They may be initial **nausea and vomiting**
- The oculomotor muscles are affected and the patient may have **diplopia and drooping eyelids with a squint**. There may be vertigo and blurred vision
- There is progressive descending motor loss with flaccid paralysis but no loss of consciousness or sensation, though weakness & sleepiness are often described
- The patient is **thirsty, with a dry mouth and tongue**
- **Difficulties in speech and swallowing**, with later problems of breathing
- There may be **abdominal pain** and restlessness
- Death is due to respiratory or cardiac failure

g) Laboratory Diagnosis

- The diagnosis may be confirmed by demonstration of
 - Organism and/or its toxin may be detected in the patient's stool or gastric contents
 - Organism and/or its toxin may be detected in the suspected food
 - Toxin may be demonstrated in the patient's blood
- Samples of vomit or faeces may also yield such evidence
- Food or stool specimens are emulsified, heated at 80°C & inoculated on blood agar
- Gram stain of the suspected colonies revealed that the organism is gram positive bacilli, motile, and sporulated. The spores are oval and sub-terminal