Clostridium

UNIT-2

Course Outcomes

- On completion of this course, the students will be able to: Apply knowledge on pathogenesis and diagnosis of Anthrax, Enterobacteriaceae and Clostridium.

Course Objectives:

- Introduction
- Classification
- Pathogenesis
- Lab diagnosis
- Treatment

Clostridium

Characteristics

• Gram-positive. obligate anaerobes capable of producing endospores which protect them in harmful environment.

• Individual cells are rod shaped.

• The spores are usually wider than the rods, and are located terminally or sub terminally.

• Most clostridia are motile by peritrichous flagella. while others have a capsule like *Clostridium perfringens*

Most Common Species

Clostridium consists of around 100 species that

- include common free-living bacteria as well as important pathogens.

There are four main species responsible for disease in humans

1. *C. perfringens*: gas gangrene; food poisoning
2. *C. tetani*: tetanus
3. *C. botulinum*: botulism
4. *C. difficile*: pseudomembranous colitis

SHAPES OF CLOSTIDIA

❖ The shape an position of spores varies in different species and is useful the identification of Clostridia

SPORES

The shape & position of spores varies in different spp. & thus useful in their identification. Spores may be;

- ✓ **Central or equatorial** in *Cl.bifermentans* (Spindle shaped)
- ✓ **Sub terminal** in *Cl.perfringens* (club shaped)
- ✓ **Oval or terminal** in *Cl.tertium* (resembling tennis racket)
- ✓ **Spherical and terminal** in *C.tetani* (drum sticks)

4

Spherical Central

Oval Central

Spherical sub-terminal

Oval sub-terminal

Spherical terminal

Oval terminal

C. perfringens

- Cultivated by Achalme 1891.
- First described in detail by Welch and Nuttal 1892.
- C. perfringens is a relatively large Gram- positive short fat bacilli with blunt ends.
- It is capsulate
- Non-motile.
- Anaerobic.
- 4 - 6µm × 1 µm
- Spore formation
- It grows quickly on laboratory media on blood agar (B – Haemolytic)

Classification

- Cl. Perfringens produce at least 12 toxins.
- But on the basis of production of four major toxins (alpha, beta, epsilon and iota).
- Classified in to five types A to E

1. Type A strains produce alpha toxin
2. Type B strains produce alpha, beta and epsilon toxins.
3. Type C strains produce alpha, beta toxin.
4. Type D strains produce alpha and epsilon toxins.
5. Type E strains produce alpha and iota toxins

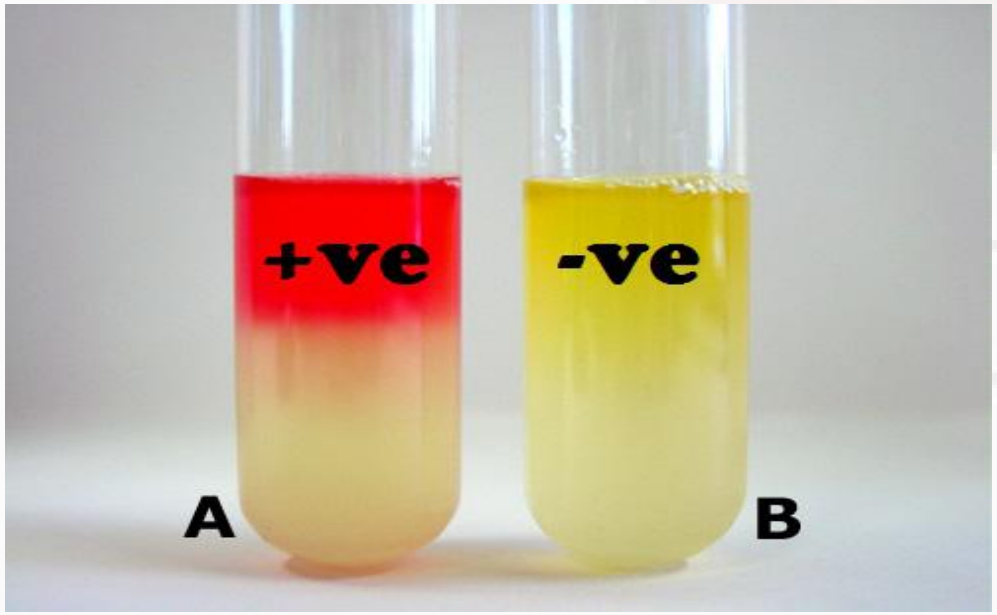
- Type A causes gas gangrene and food poisoning in man

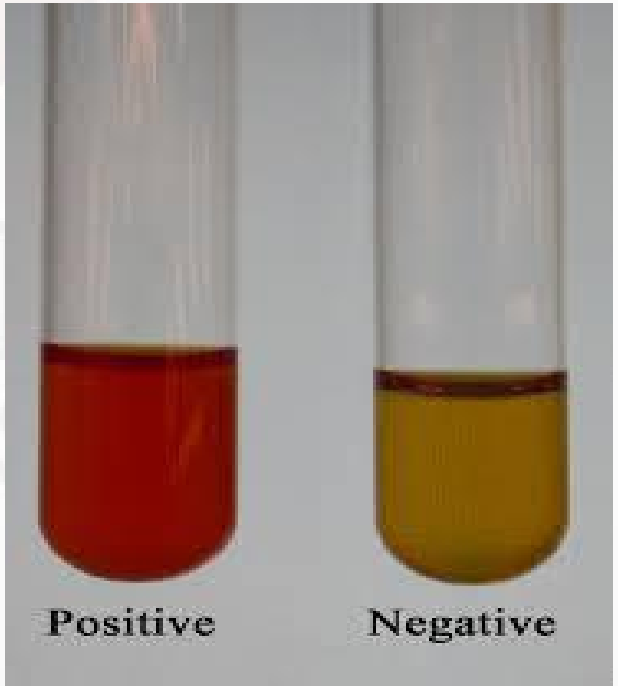
Culture:

- Blood Agar, Cooked meat broth (CMB) and thioglycollate broth
- It is Anaerobic
- pH range of 5.5 - 8.0
- Optimum temperature for growth 37^{o}C.
- Complete haemolysis caused by theta – toxin
- Incomplete haemolysis due to alpha toxin

Biochemical Reaction

- It ferments glucose, lactose, sucrose and maltose with Acid and Gas
- Indole negative
- MR positive (**Methyl Red)**
- VP negative (**Voges–Proskauer)**





Alpha Toxin

- It is produced by all types of Cl.perfringens.
- It is phospholipidase and responsible for profound toxaemia in gas gangrene.
- The toxin is haemolytic for red blood cells of animal species.
- Nagler reaction

Cl.perfringens Produce diseases

- The organisms associated **with gas gangrene** attack soft tissues by producing toxins and aggressins, and some strains of **the bacteria** produce enterotoxins and cause food poisoning

Collecting a Infected Tissues

- If there are pieces of necrotic tissue in the wound, small pieces should be transferred aseptically into a sterile screw-capped bottle and examined immediately by microscopy and culture.
- Specimens of exudate should be taken from the deeper areas of the wound.

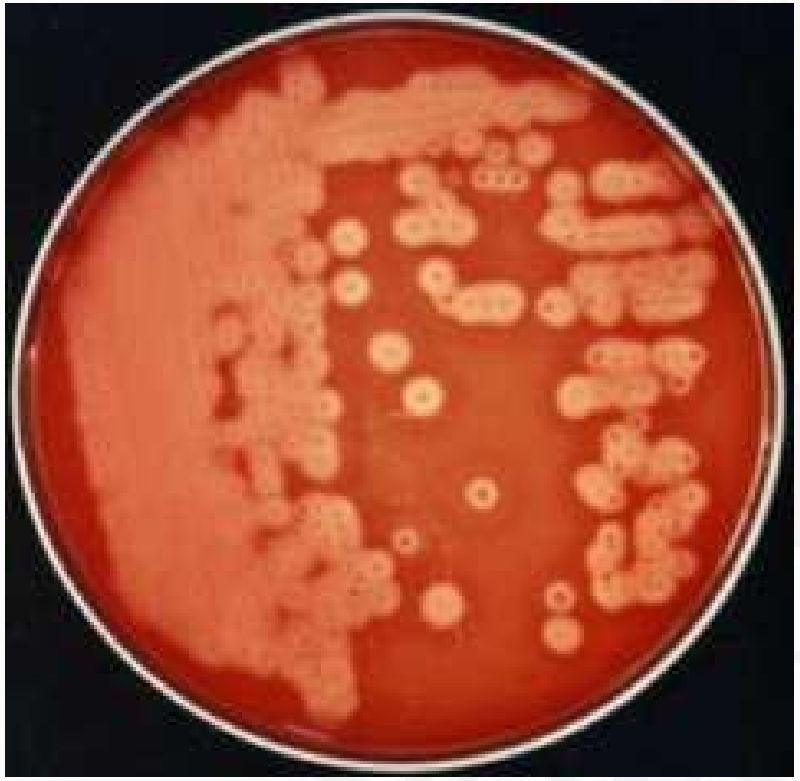
Media used for Cultivation

- Liquid medium for cultivation cooked meat broth

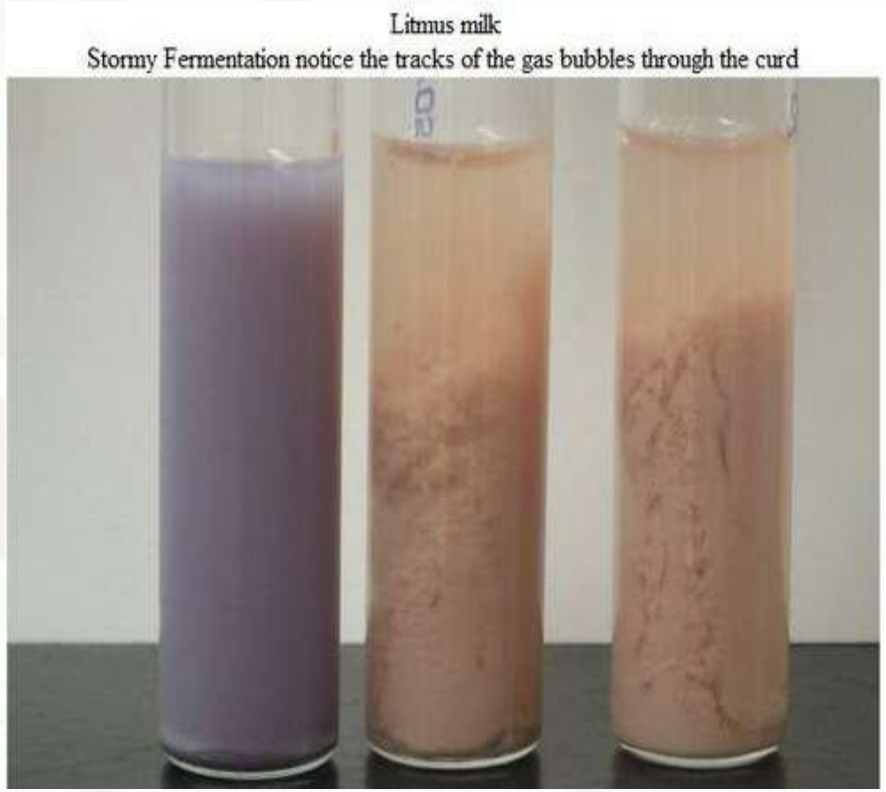
- Thiglyclolate broth
- CMB contain unsaturated fatty acids which take up oxygen

Clostridium. perfringens

Culture & identification



B – Haemolysis
On blood agar media



1= Control, 2 and 3= "Stormy clot fermentation"
Clostridium perfringens will produce acid and gases

Litmus Milk Test

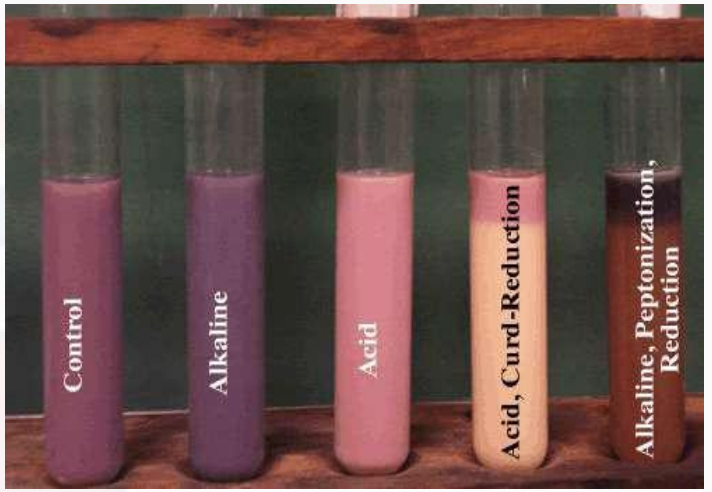
It distinguish between different species of bacteria.

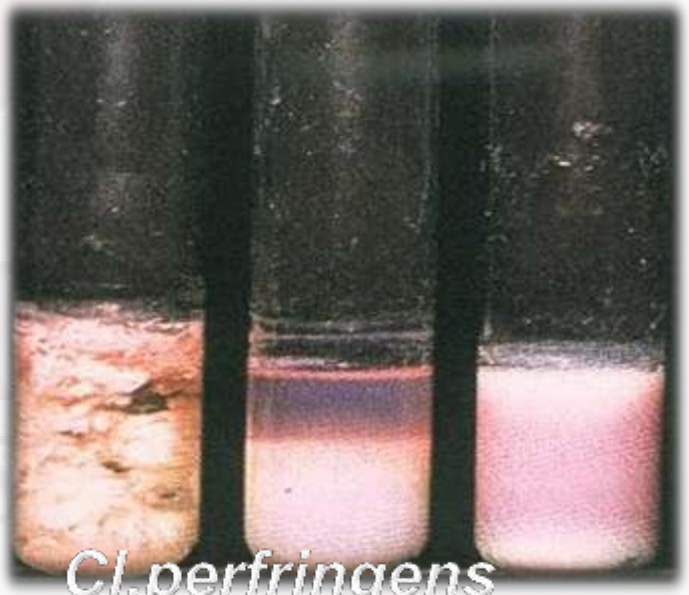
The lactose (milk sugar), litmus (pH indicator), and casein(milk protein) contained within the medium can all be metabolized by different types of bacteria.

Milk is the first substrate used to

- maintain bacteria, this test allows for accurate depiction of bacterial types.

• The addition of litmus, other than explaining the pH type, acts as an oxidation-reduction indicator. The test itself tells whether the bacterium can ferment lactose, reduce litmus, form clots, form gas





Nagler's Reaction

(Lecithinase test)

- **Naglers** test is important for the identification of alpha toxin of Clostridium perfringens, the addition of antitoxin to one half of egg yolk agar prevents visible opacity, due to lecithinase action which is normally observed around colonies. Other species of Clostridium shows Negative **Naglers reaction**

- The bacteria is streaked on the medium containing egg
- yolk (contains lecithin)
- The plate is incubated anaerobically at 37 C for 24 h
- 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 antitoxin is present on the medium

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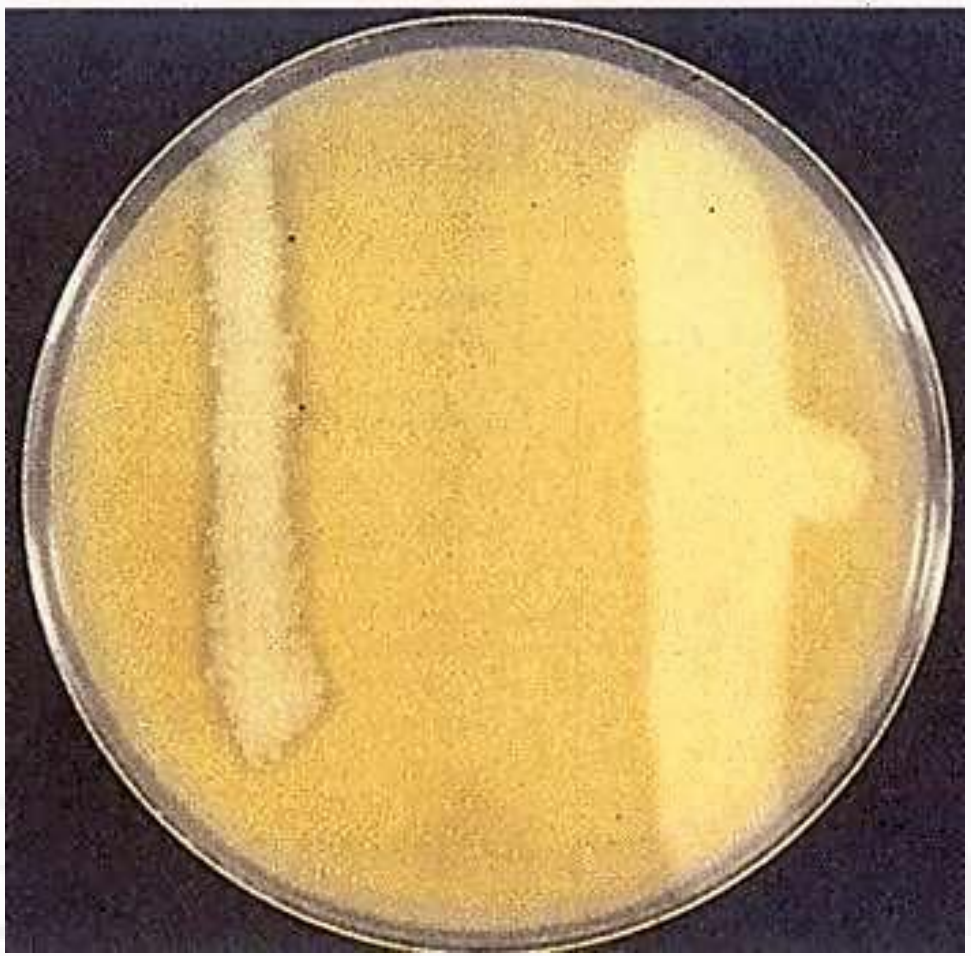
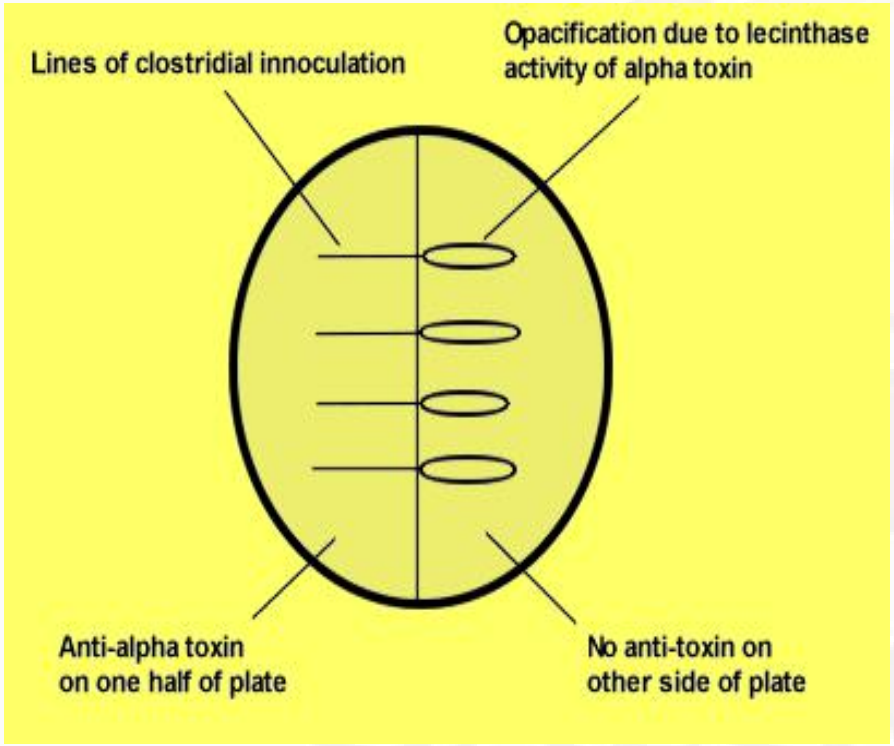
C. perfringens Nagler Reaction

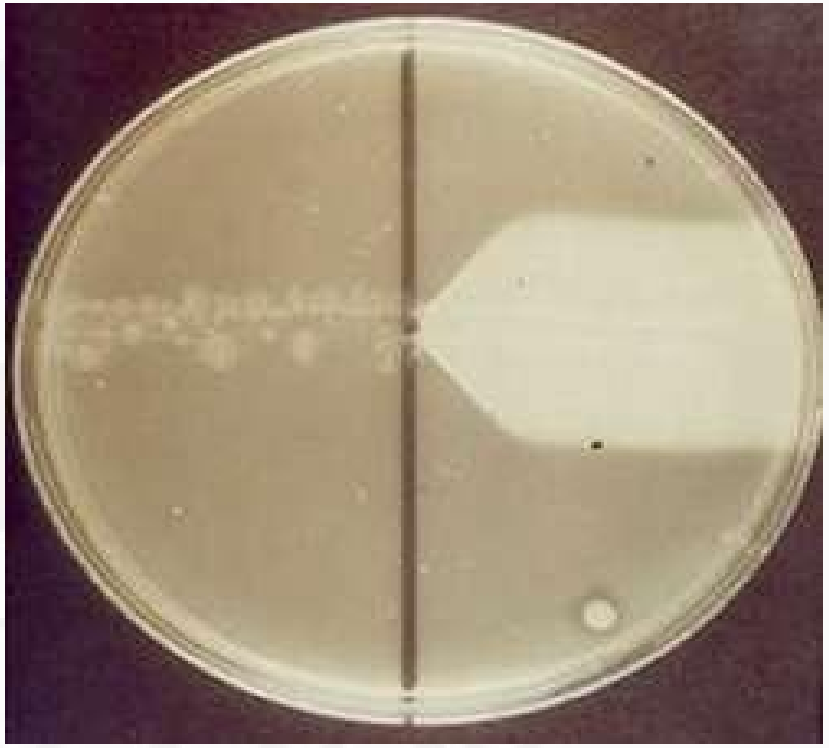


NOTE: Lecithinase (α-toxin; phospholipase) hydrolyzes phospholipids in egg-yolk agar around streak on right. Antibody against α-toxin inhibits activity around left streak.

Nagler Reaction



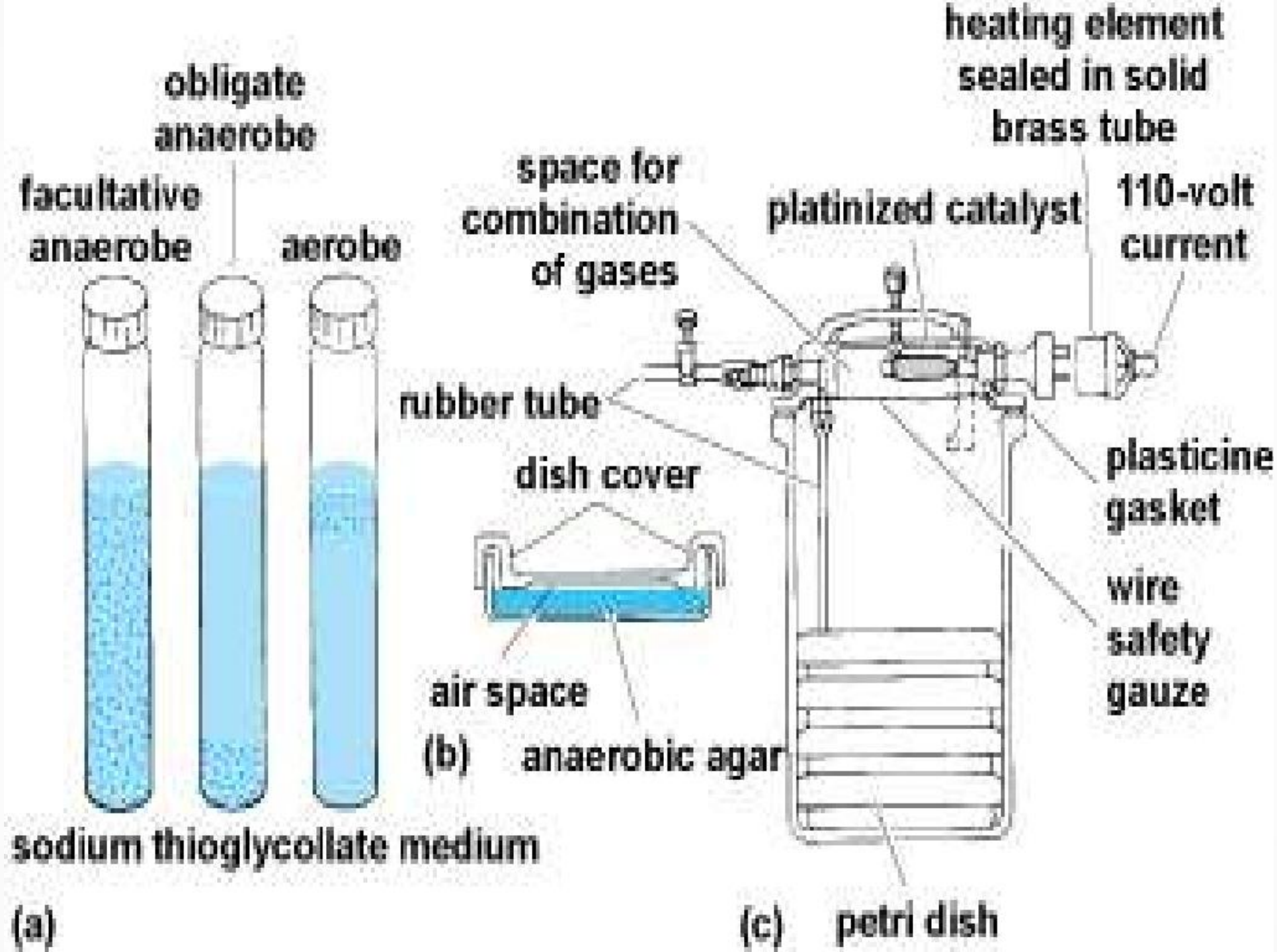
Procedure of Nagler Reaction



Positive Nagler Reaction

Specific Requirements for Anaerobic Cultivation

Anaerobic Jar


obligate anaerobe
facultative anaerobe
aerobe
sodium thioglycollate medium
(a)
space for combination of gases
rubber tube
dish cover
air space
anaerobic agar
(b)
heating element sealed in solid brass tube
platinized catalyst
110-volt current
plasticine gasket
wire safety gauze
petri dish
(c)

Clostridium tetani

Clostridium tetani

Anaerobic bacteria of the genus species *Clostridium* it is gram positive, slender bacillus and it has spherical terminal spores giving drum stick appearance

It is non capsulated & motile with peritrichus flagella

It produces a potent biological toxin, tetanospasmin, and is the causative agent of tetanus a disease characterized by painful muscular spasms that can lead to respiratory failure and, in up to 40% of cases, death.

- Clostridium tetani is the causative agent of tetanus. Clostridium tetani is widely distributed in soil and intestine of man and animals.

- **Morphology:**
- Gram positive, slender bacillus
- 4-8 µm × 0.5 µm.
- Terminal spores formation.
- Drumstick appearance
- Non – capsulated
- Motile with peritrichous flagella.
- Anaerobic bacteria

Culture

- Grows on ordinary media.
- The optimum temperature for growth is $37^{o}C$
- pH 7.4
- Grow well in cooked meat broth and thioglycollate broth and Blood agar
- Produce a swarming (thin spreading film) on blood agar.
- produce Alpha haemolytic colonies

Biochemical Reaction

- Indole Positive
- MR and VP negative.

Classification

- The serological types (I to X) of Cl. tetani.

Toxins

- Tetanolysin (Hemolysin)
- Tetanospasmin (neurotoxin)

What is Tetanus?

- An infectious disease caused by contamination of wounds from the bacteria ***Clostridium tetani***, or the spores they produce that live in the soil, and animal feces

- Infection follows **when spores**
- **become activated and develop**
- into gram-positive bacteria that multiply
- and produce a very powerful toxin (**tetanospasmin**) that affects the muscles.

Tetanus spores are found throughout the environment, usually in soil, dust, and animal waste.

Tetanus is acquired through contact with the environment; it is not transmitted from person to person.

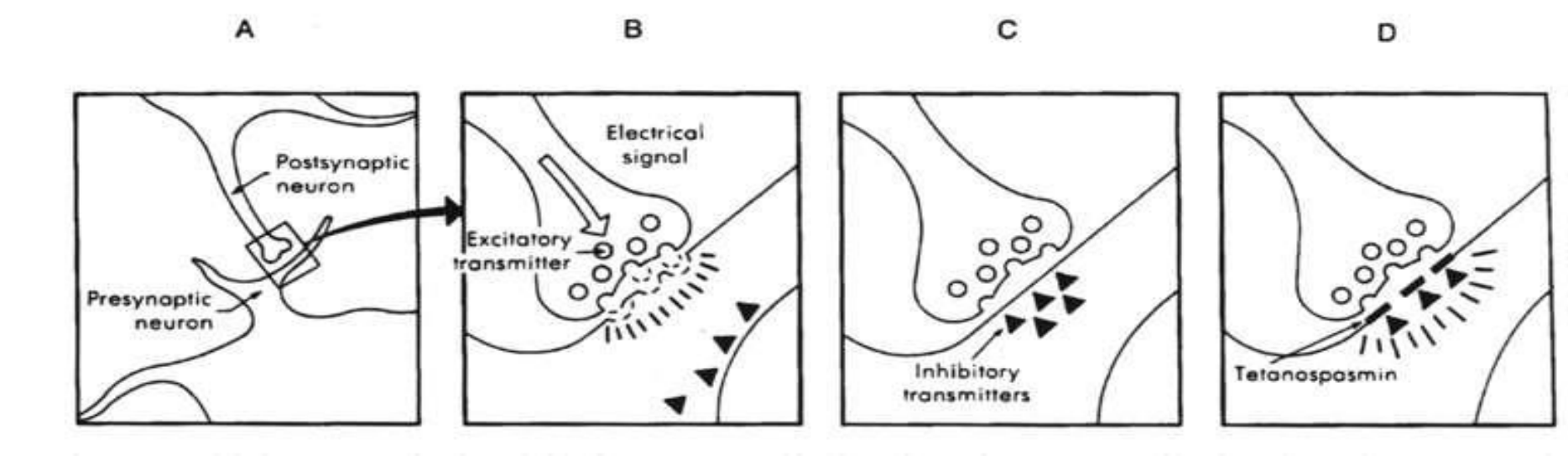
Causes

- The usual locations for the bacteria to enter the body:
  - Puncture wounds (such as those caused by rusty nails, splinters, or insect bites.)
  - Burns, any break in the skin, and IV drug access sites are also potential entryways for the bacteria.

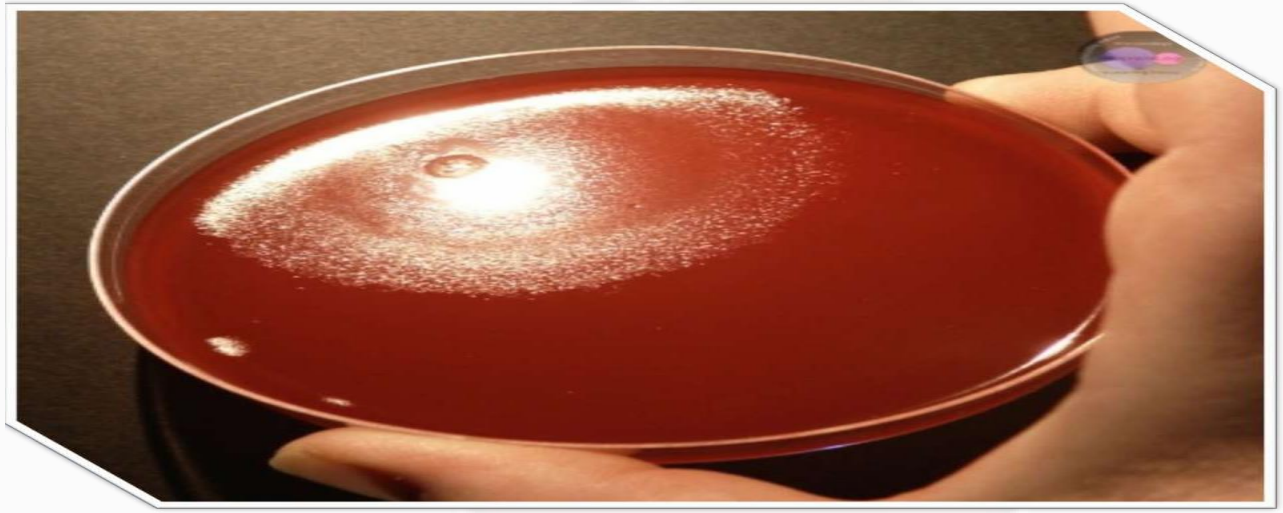
Mode of action of tetanospasmin

1. It inhibits the release of acetylcholine thus it interferes with neuromuscular transmission.

2. Inhibition of postsynaptic spinal neurons by blocking the release of an inhibiting mediator



Laboratory Diagnosis:



- Gram +ve stains grow on blood agar media aerobically
- Inoculation of culture in to 2 mice one is protected with anti-toxin and the other is unprotected (dies with typical tetanic spasms)

Clostridium difficile

CLOSTRIDIUM DIFFICILE

- Cl. difficile was first isolated from the faeces of newborn infants.
- It was so named due to unusual difficulties involved in its isolation.

Morphology

- It is a long, slender.
- Gram positive bacillus containing oval and terminal spores.
- **Toxins:**
- Enterotoxin (toxin A)
- Cytotoxin (toxin B)
- The enterotoxin is primarily responsible for diarrhoea.
- Cytotoxin capable of producing cytopathogenic effects in several tissue culture cell lines.

Pathogenesis

- The pathogenic role of Cl.difficile is established in 1977.
- Pseudomembranous colitis.
- Acute colitis

• Laboratory Diagnosis:

- Demonstration of toxin by ELISA.
- Cycloserinecefoxitin-fructose agar (CCFA).
- C.difficile colonies appear yellow due to fructose fermentation.

• **Treatment**

- Vancomycin
- Metronidazole

Clostridium Botulinum

- Clostridium botulinum causes a severe form of food poisoing named botulism.
- The term botulism means sausage (botulus, Latine for sausage)
- Present in soil, animal manure and sea mud.

• Morphology

- It is a Gram positive bacillus
- about 5 µm × 1 µm.
- Motile by peritrichate flagella
- Produces subterminal , oval, bulging spores.

Culture

- Anaerobic
- Optimum temperature 37^{o}C
- Blood Agar (Haemolysis) and Cooked meat broth.
- Colonies are large, irregular, semitransparent.

- Classification

- Eight types
- A, B, C1, C2, D, E, F and G
- Neutralized by Homologous antiserum.

Toxins

- Exotoxin
- Produced intracellularly .
- Protoxin is converted to active toxin by trypsin and proteolytic enzymes.
- The toxin acts by blocking production.
- Release of acetylcholine at synapses and neuromuscular junction.

• Pathogenesis

- Botulism is of three types

1. Food borne
2. Infants botulism
3. Wound botulism

Pathogenesis

- **Foodborne Botulism:**

- Caused by types A, B, E and C, F and G.
- Symptoms appear 12-36 hours after ingestion of contaminated food.
- Vomithing
- Thirst
- Constipation
- Ocular paresis
- Difficulty in swallowing.

- **Infants Botulism**

- Below 6 months
- Due to ingestion of food contaminated by spores.
- Honey is the source of infection.
- Weakness, paralysis and loss of tone.

Wound botulism

- wound infection.
- symptoms similar to foodborne botulism.

• Laboratory Diagnosis:

- stool, food and vamitus.
- Gram Staining
- Toxin identification.

• Treatment

- Penicillin and metronidazole
- Vaccine

OBJECTIVE QUESTIONS

Apply your knowledge on Tetanus

Explain clostridium classification

Discuss on lab diagnosis of Clostrodium species

Video links

- https://www.youtube.com/watch?v=h_A8yVY1kBg
- https://www.youtube.com/watch?v=6astF1cU5SU
- https://www.youtube.com/watch?v=HKskJ4JMg40

Reference

- 1. Logeswari Selvaraj, textbook of microbiology for paramedicals, jaypee publication.
- 2. Prescotts, microbiology,8th Edition.

THANK YOU