

Clostridium

Anaerobic gram positive bacilli – capable to form spores

- Saprophytes in soil
- Commensals in Intestinal tract of human and animals
- Rare Commensals in vagina
- Species:
- -Clostridium perfringens- Gas gangrene
- -Clostridium tetani- tetanus
- -Clostridium botulinum- botulism
- -Clostridium difficile- pseudomembranous colitis

Pathogenicity:

- depending upon environment provided in body / tissue – grow in anaerobic environment – produce powerful toxins – disease
- Gas gangrene
- Purpural infection
- Septicemia
- Enteritis
- Tetanus
- Food poisoning (botulism)
- Pseudomembranous colitis

Gas gangrene

- Defined as a rapidly spreading. Edematous myonecrosis, occurring characteristically in association with severe wounds of extensive muscle masses that have been contaminated with pathogenic clostridia particularly *C. perfringens*
- Such conditions arises in wars, road accidents or other type of injuries involving crushing of large muscle mass and contaminated with soil.

Clinical presentation

- Incubation period: 7 hours to few weeks (average 1 to 6 days)
- Local effect: Due to multiplication of bacteria, gas production, effect of toxins – pain, sero sanguinous discharge, Tenderness , edema, crepitations, gangrene
- Systemic – due to profound toxemia – circulatory failure , death.

Disease - tetanus

- Results from contamination of wound which are vulnerable (particularly puncture wound) and favors the growth of anaerobic bacteria
- May be asso. With some unhygienic practice – application of cow dung on umbilical stump (tetanus neonatarum, ear boring, circumcision)
- Also asso. With otitis media, septic abortion.

Tetanus – favorable conditions

Mere contamination will not lead tetanus, following conditions favors,

- Destruction and necrosis of tissue
- Lack of drainage in area
- Presence of extraneous material
- Infection with other bacteria

Spores may remain dormant until favorable conditions provided

Tetanus – clinical presentation

- Incubation period 2 days to several weeks.
- First symptoms usually trismus
- Followed by generalized spasm
- Posture of body ad type of convulsion acc. To power of muscle
- If untreated – mortality – 80 – 90 %
- In timely treated case – 15 – 20 % mortality

Botulism

- Food borne: usually by consumption of preserved food – meat products, canned vegetables, fish;
- - symptoms begins 12 – 36 hours after food consumption – vomiting, constipation difficulty in swallowing, breathing, death due to respiratory paralysis
- Wound botulism, infant botulism - rare

Cl. difficile

- Antibiotic associated colitis
- Pseudo membranous colitis
- Asso. With long indiscriminate use of antibiotics – lead killing of majority of Commensals and survival of Cl difficile (Resistant to majority of antibiotics) – over grow – produce toxins – colitis
- Treatment : Vancomycin, Metronidazole

Laboratory diagnosis

❖ Specimen:

- Pus or infected tissue from wound for microscopy and culture
- -blood, vomit and left over food for detection of toxin if botulism is suspected
- -feces and food for culture if food poisoning due to *Cl.perfringens*/*Cl. Botulinum* is suspected

❖ Direct method of demonstration of organism:

-Morphology: Clostridia are spore forming gram positive rods.

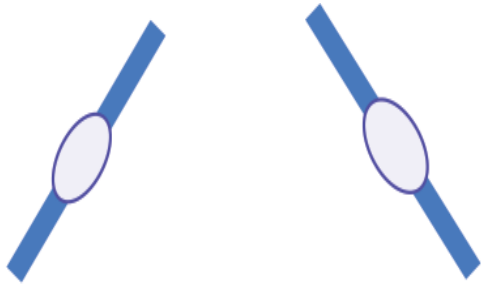
➤ Cl.perfringens: Non-motile, capsulated, thick, brick shaped ,Box car shaped rods .

May have central or subterminal spores which are rarely seen from tissue and direct smear from sample.

➤ Cl.tetani: Motile, non capsulated, long thin with round spore at one end, drum stick appearance

➤ Cl. botulinum: Motile pleomorphic rods with sub terminal spores.

Spores of Clostridia



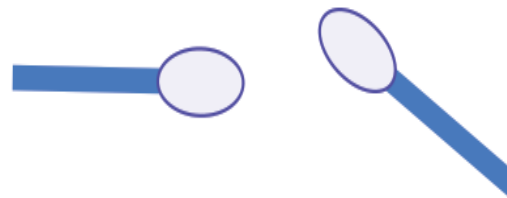
Oval and central spores
(e.g. *Clostridium bifermentans*)



Subterminal spores
(e.g. *Clostridium perfringens*)



Spherical and terminal spores
(e.g. *Clostridium tetani*)

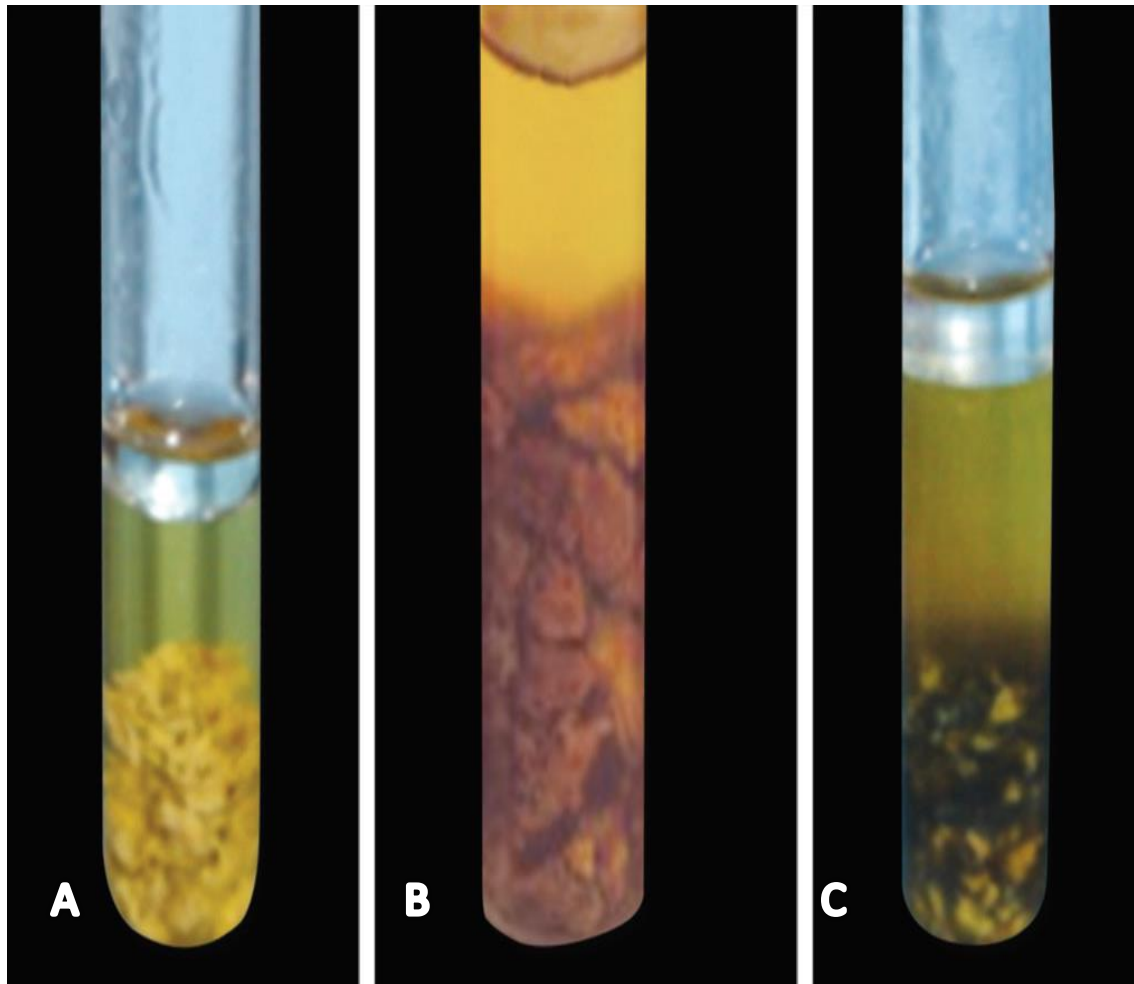


Oval and terminal spores
(e.g. *Clostridium tertium*)

Cultivation

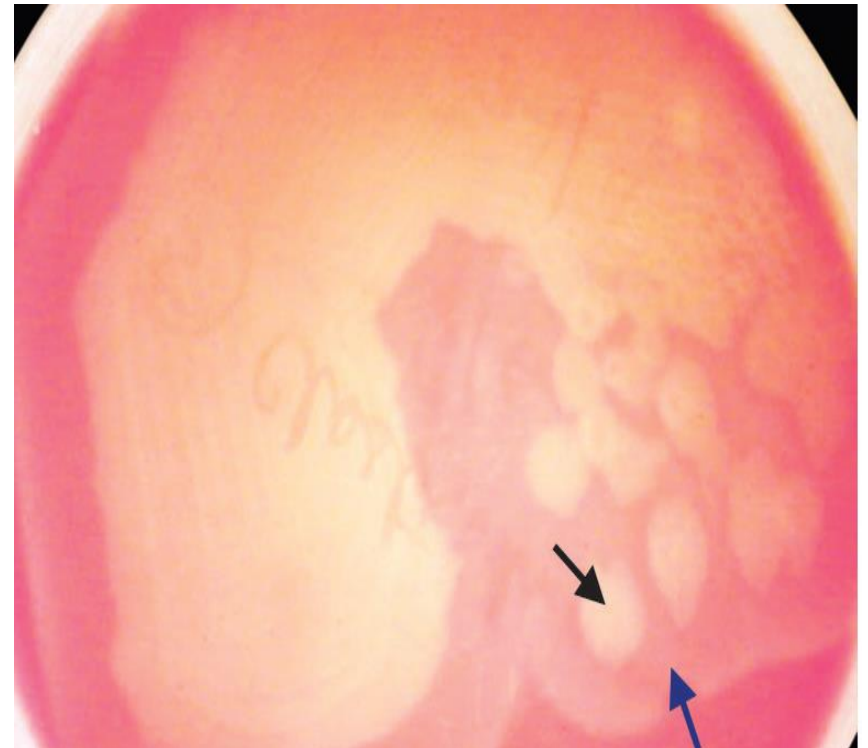
- Clostridia grow well in common anaerobic media
- **Robertson's cooked meat (RCM) broth**
 - Chopped meat particles → glutathione and unsaturated fatty acids which take up oxygen
- Proteolytic - turn the meat black and produce foul odor, e.g. *C. tetani*, *C. botulinum A, B and F*.
- Saccharolytic species - turn the meat pink, e.g. *C. perfringens*, *C. difficile* and *C. botulinum C, D and E*.

**Robertson cooked meat broth: A. Uninoculated;
B. Pink and turbid (*C. perfringens*); C. Black and turbid (*C. tetani*)**



Blood agar

- *Cl.perfringens*:
- Double zone hemolysis
- Blood agar - inner narrow zone of complete hemolysis (due to θ toxin), surrounded by a much wider zone of incomplete hemolysis (due to the alpha toxin)



-Cl. tetani: Fine film of growth is produced on surface of blood agar.

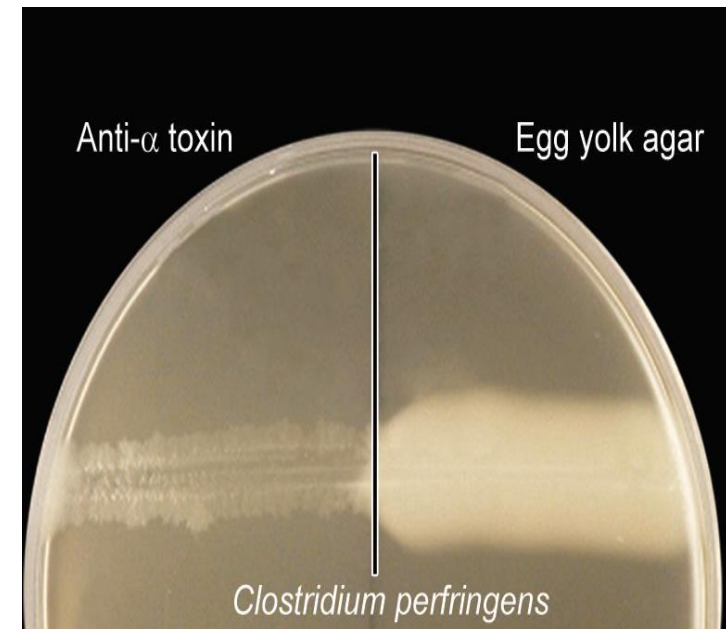
-Cl. Botulinum: some strains produce beta hemolysis. Semi transparent, large colonies with wavy outline.

Biochemical reactions:

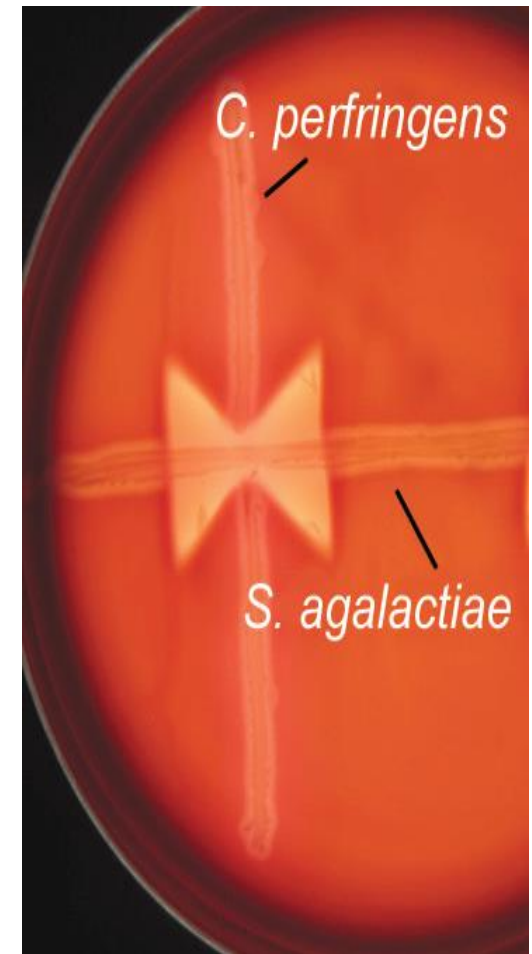
Species	Lecithinase C activity	Lipase hydrolysis	Lactose fermentation	Proteinase activity
Cl.Perfringens	+	-	+	-
Cl. Tetani	-	-	-	-
Cl.botulinum	-	+	-	+

Lecithinase activity of α toxin

- **Opalescence** surrounding streak line on egg yolk agar or media containing 20% human serum
- Opalescence inhibited by anti- α toxin if added in medium
- **Positive** – *C. perfringens*, *C. Bifermentans*, *C. baratti* and *C. sordellii*



- ***C.perfringens*** is streaked over the center of blood agar plate and ***Streptococcus agalactiae*** is streaked perpendicular to it
- Presence of **enhanced zone of hemolysis** (arrow-shaped) pointing towards *C.perfringens* indicates the test is positive(CAMP Test).



Animal inoculation

Organism	Animal	Site
Cl.Perfringens	Guinea pig	Intramuscular
Cl. tetani	Mice	s/c into the base of tail
Cl.botulinum	Mice	Intraperitoneally