3) GPB Clostridium:

Clostridium is a GPB. which is.

i) Anaerobic.

ii) Bulging spore producing bacteria

pathogenic sps:

- Cl. perfringens & Welchi. ⇓ Gas gangrene. → Club shaped
- Cl. tetani. ⇓ Tetanus. → Drumstick. → Terminal (1) bulging
- Cl. botulinum ⇓ Botulinism → Club shaped
- Cl. Difficile ⇓ Pseudo-membranous Colitis. → Club shaped.

Sub Terminal bulging (3).

only Capsulated Clostridium among these (4)

Virulence factors:

(i) Capsule: Anti phagocytic

(ii) Toxins: α, β, ...

most imp. α-Toxin ⇒ why?

Lecithinase activity. (phospholipase).

Clinical manifestatn:

i) Cellulitis —will lead to→ myonecrosis. (death of m/s)

cellulitis.

Ischaemia

myonecrosis

platelet Aggregatn → Ischaemia

due to Abundant Gas prodn., Myonecrosis Caused by this is called Gas Gangrene.

ii) Food poisoning

* For all poisoning due to Toxin we must find whether the toxin is pre/post-formed then we can Conclude the Incubatn period.

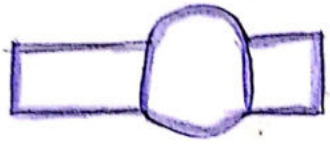
* α-Toxin ⇒ post-formed.

→ Source: Meat & vegetables.

Selective medium: RCM (Robertson Cooked Meat).

ii) Microscopy:

Gram stain (G/s). →



↑
Sub Terminal Bulging Spore
↓
club shaped App.

iii) Toxin demonstration:

α- Toxin. → Hemolysin. ✓
→ Lecithinase ✓.

⑤ methods of demonstrat.

① Naeglar's Rxn.
② Reverse CAMP Test
③ Double zone Hemolysis

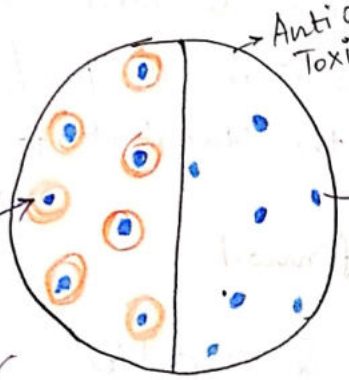
① Naeglar's Rxn:

* To demonstrate lecithinase of α-Toxin.
* Egg Yolk. Agar → Divide into ②.

Add Anti α-Toxin to one ½

Apply Anaerobic culture methods. (Mc Intosh Fidle's Jar)

Add clostridium



opalescence ↓ due to lecithinase activity of α-Toxin. (egg yolk → lecithin)

α-toxin + Lecithinase → Opalescence.

b) Reverse CAMP Test:

↓ demonstrate Hemolysin prop. of α-toxin.

* Blood Agar is Taken. (RBCs).

↓

~~Divide into 2~~

↓

put Group-B Strepto. on ~~one half~~ midline.

↓

Grp-B Strep → CAMP factor (Hemolysin)

↓

Streak. Clostridium perpendicular to. Grp-B' Strepto.

↓

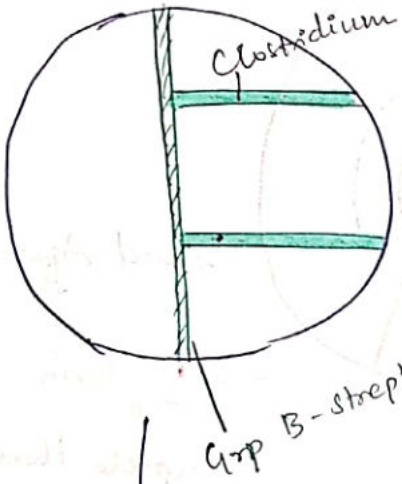
Hemolysis Takes place due to
i) CAMP
ii) α-Toxin.

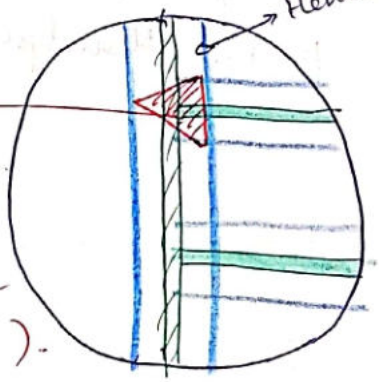
↓

∵ Both are Synergising the area where both meet, Hemolysis is increased

↓

ARROW HEAD Hemolysis formed





Arrow head Hemolysis (synergistic action of CAMP + α).

Note: No other Bacteria produce this Arrow head Hemolysis.

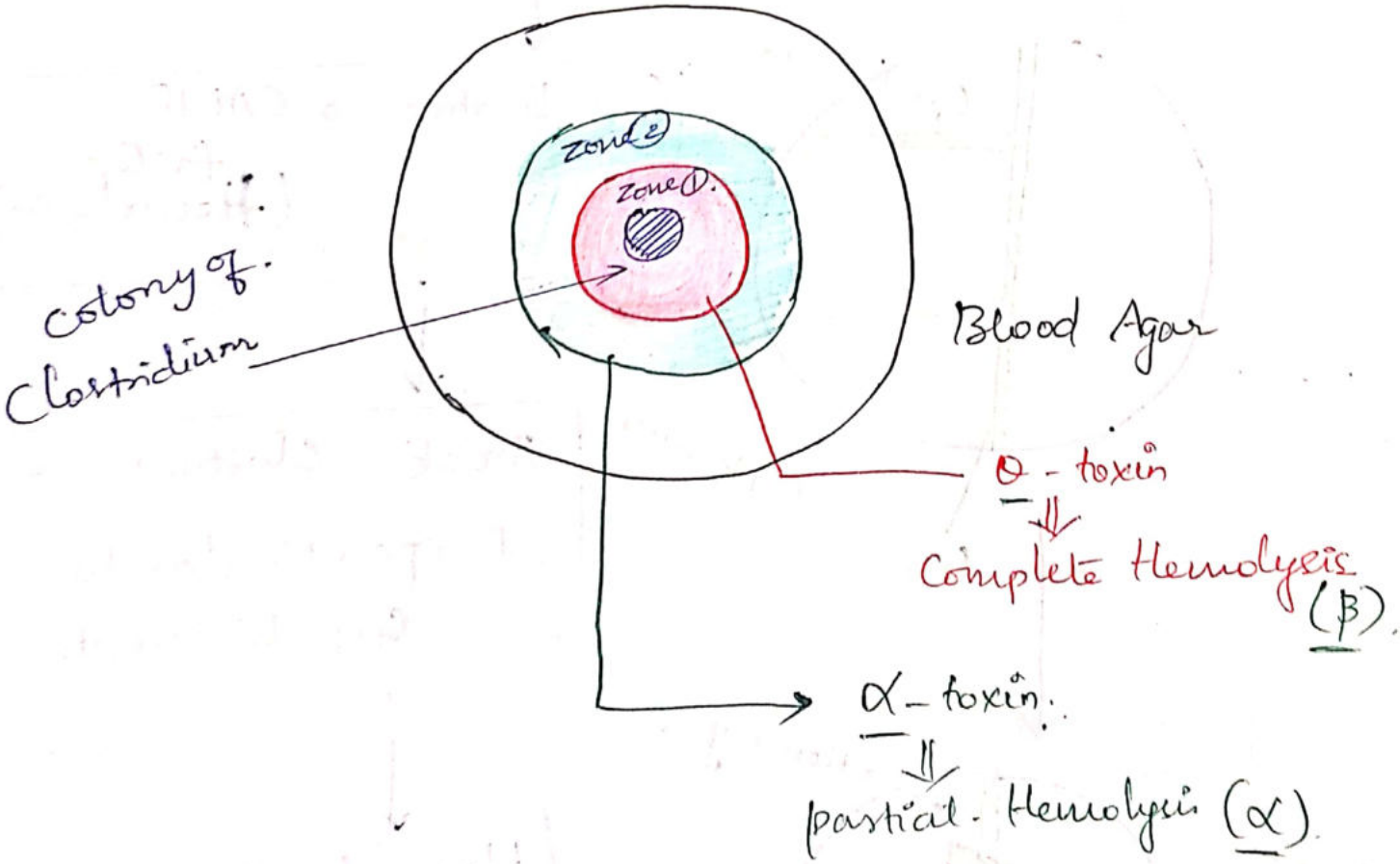
c) Double Zone Hemolysis : (OR) Target Hemolysis

↓ ↓

(2) Zones of partial & Complete Hemolysis are formed.

Target (◎) Shaped Hemolysis pattern is formed

* To demonstrate Hemolytic activity of Toxins (α & θ).



Virulence Factors:

i) Toxins

- Tetanolysin
  - ⇓ Causes Host Cell lysis.
- Tetano-Spasmin
  - ⇓ Causes Spastic paralysis. (Tetanus).

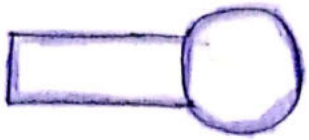
Clinical manifestatn:

- Lock Jaw
  - Unable to open mouth
  - ↑ Tetanus of masseter.
- Tetatius.
  - Tetanospasmin.
    - ⇓ ⊖ the ⊖ NeuroTransmitter (GABA)
      - ⇓ (Continuous m/s Contracⁿ)
        - ⇓ Spastic paralysis. (or) Tetanus.

(i) Culture: RCM.

(ii) Microscopy:

G/S →



↑ Terminal spores. · bulging.

⇓

drum-stick app.

(iii) Toxin Demonstratn:

(In vivo).

↳ "inside lab animal."

Take (2) ~~mouses~~ mice:

Mouse ①.	Mouse ②.
* Add Anti Tetanus Toxin	* — X —
* Then sample / Cl. Tetanus is added.	* Cl. Tetanus sample is added.
* No paralysis	* Spastic paralysis.

⇓

Tetanus Toxin is demonstrated

Both culture & microscopy are almost same for all Clost. so Toxins should be demonstrated Correctly ID...

Clostridium Botulinum :

Virulence factors :

TOXIN: Botulinum Toxin (8 Types).

8 - A_1, B, C_1, C_2, D, E, F, G.

C_2 - entero Toxin

* causes food poisoning
* preformed. (I.P is less)
* Source: meat & vegetables

other (7) - NeuroToxins

* causes flaccid paralysis.
* post formed.

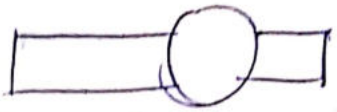
How Neurotoxins cause flaccid paralysis ?

Neurotoxins
↓
⊖ ACh
↓
m/s can't contract
↓
flaccid paralysis.

ACh - Acetyl choline
m/s - muscles

Dis :

i) Culture : RCM.

ii) Microscopy : G/S →  ⇒ Club shape

Sub Terminal Bulging spore.

iii) Toxin Demonstration:

(IN-vivo).

Take ② mice.

mouse ①	mouse ②
* Inject Botulinum ANTI-toxin	* — Nil —
* Sample of Cl. Bot. is added	* Sample ---- --- added.
↓	↓
No paralysis (bcoz Antitoxin destroys Botul. Toxin)	Flaccid paralysis.

Virulence Factors:

actually a Commensal in Intestine

but due to some predisposing conditions it can turn pathogenic.

Commensal —(long Term A.b. use.)→ Pathogen (acquires virulence factors).

Long Term use of Ab. such as.

Ceftriaxone > clindamycin > Ampicillin

↓

Kills other commensal other than Cl. Difficile

↓

So Cl. Difficle now can grow more & becomes pathogenic.

↓

acquires Virulence factor.

VIRULENCE FACTORS.:
TOXINS (2).

- Exotoxin A
- Exotoxin B.

↓

Clinical manifestation

Exotoxin A & Exotoxin

↓

clinical manifestation → Diarrhoea & pseudomembranous Colitis

---

Δsis:

i) Culture: (stool culture).

selective med.: CCFA → Cefoxitin, Cycloserine, Fructose Agar

ii) Microscopy:

G/s- 

Sub Terminal Bulging spore ⇒ Club shaped.

iii) Toxin Demonstration: In-vitro.

② methods.

Molecular (best method) → PCR has High accuracy

↓

PCR

↓

detect Toxin genes.

Immunological.

↓

ELISA / Rapid Test.

↓

detect Toxins itself.

Note:

if an org. grows on select
cl. difficile then its not loo
Thats why we demonstr
which proves that its
Also Non Pathogenic (Commensal) C