



**AZERBAIJAN MEDICAL UNIVERSITY**  
**DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY**

**Lesson 5.**

**Microbiology diagnosis of diseases, caused by anaerobes  
(clostridium and bacteroides)**

**FACULTY: General Medicine**  
**SUBJECT: Medical microbiology - 2**

## Discussed questions:

1. The causative agent of tetanus, morpho-biological characteristics, pathogenesis of the disease, microbiological diagnosis, principles of specific treatment and prevention.
2. The causative agent of botulism, morpho-biological characteristics, pathogenesis of the disease, microbiological diagnosis, specific treatment and prevention principles.
3. The causative agents of gaseous anaerobic infections (*C.perfringens*, *C.novyi*, *C.septicum*, *C.histolyticum*, *C.sordellii*), morpho-biological characteristics, pathogenesis of the disease. Other diseases caused by *C.perfringens* (food poisoning, necrotic enteritis)
  - Microbiological diagnosis of gaseous anaerobic infections, principles of specific prevention and treatment.
4. *Clostridium difficile*, morpho-biological characteristics, pathogenesis of pseudomembranous colitis, microbiological diagnosis, prevention and treatment problems
5. Morpho-biological characteristics of bacteria from the genus *Bacteroides*, principles of microbiological diagnosis of diseases.

## Purpose of the lesson:

- To acquaint students with the morpho-biological characteristics of pathogenic anaerobes, which are the causative agents of tetanus, botulism and gaseous anaerobic infections, as well as bacteria from the genus *Bacteroides*, methods of microbiological diagnosis of diseases, principles of treatment and prevention.

# DEFINITIONS

- **OBLIGAETE ANAEROBE**
  - Lack superoxide dismutase and/or catalase
  - toxic radicals formed by oxidative enzymes kill organisms
- **AERO-TOLERANT ANAEROBES**
  - survive in presence of oxygen
  - Do not use oxygen for energy requirements
- **FACULTATIVE ANAEROBES**

# Gram-positive anaerobes

- *Actinomyces* (head, neck, pelvic infections; aspiration pneumonia)
- *Bifid bacterium* (ear infections, abdominal infections)
- *Clostridium* (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
- *Peptostreptococcus* (oral, respiratory, and intra-abdominal infections)
- *Propionibacterium* (shunt infections)

# Gram-negative anaerobes

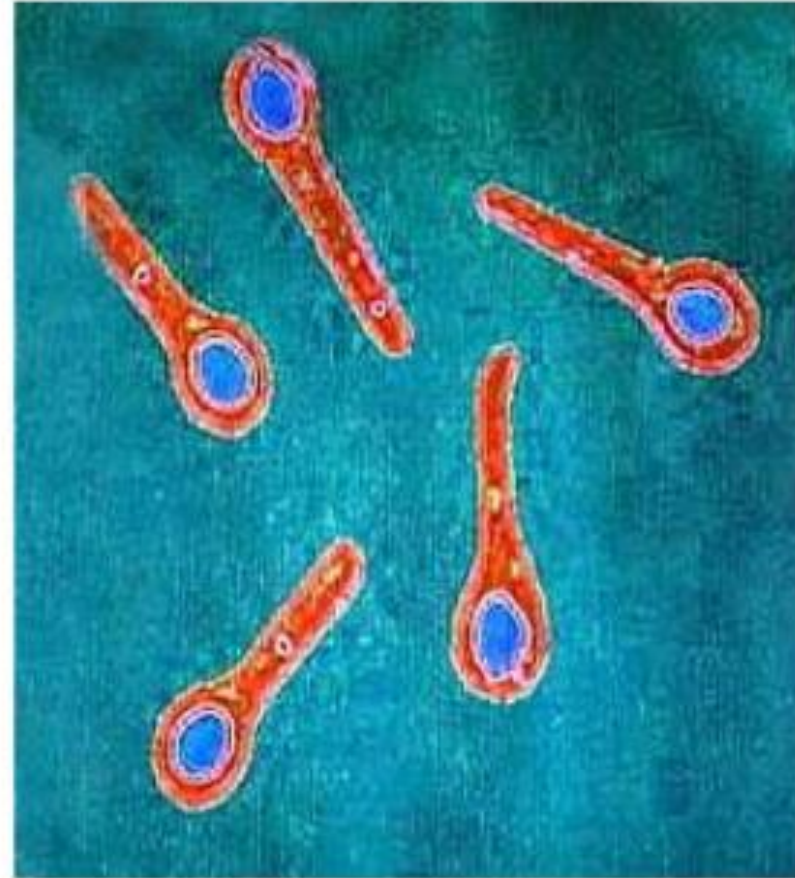
- *Bactericides* (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
- *Fusobacterium* (abscesses, wound infections, pulmonary and intracranial infections)
- *Porphyromonas* (aspiration pneumonia, periodontitis)
- *Prevotella* (intra-abdominal infections, soft tissue infections)



# CLOSTRIDIA

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- Gram positive spore forming bacilli
- ubiquitous
  - intestines of man and animals
  - animal and human faeces
  - contaminated soil and water
- Several species associated with human disease



# CLOSTRIDIUM-introduction

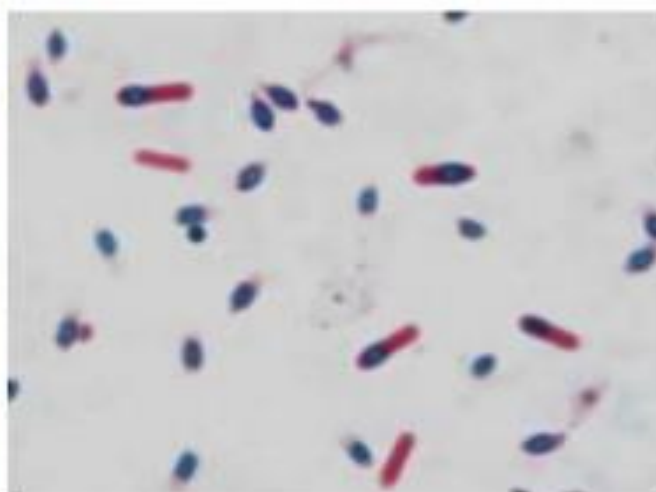
- **Clostridia:**

- are strictly anaerobic to aerotolerant sporeforming bacilli found in soil as well as in normal intestinal flora of man and animals.
- There are both gram-positive and gram-negative species, although the majority of isolates are gram-positive.
- Exotoxin(s) play an important role in disease pathogenesis.
- motile -- peritrichous flagella  
(exception: *C. perfringens*—nonmotile)
- the sporangia– swollen
- typical clinical symptoms



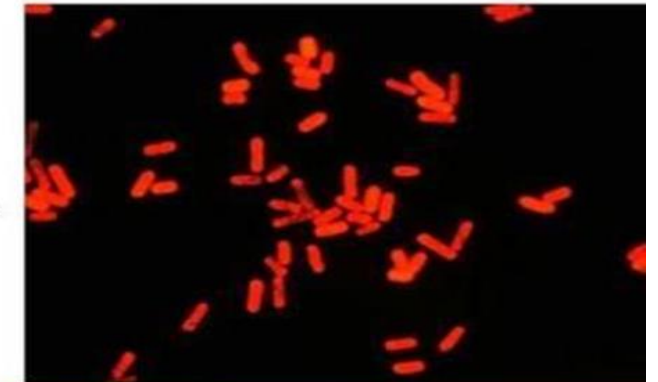
## Genus Clostridium

- The genus consists of G+ve, anaerobic, Spore forming bacilli.
- Spores are wider than bacillary body, giving bacillus a swollen appearance resembling spindle; hence named so (Kolster meaning spindle ).
- Highly pleomorphic, straight or slightly curved rods with slightly curved ends.
- G +ve , 3-8 x 0.4-1.2  $\mu\text{m}$  in size
- **Motile (except *Cl. tetani* Type VI & *Cl. perfringens*)**
- ***Cl. perfringens* & *Cl. butyricum* are capsulated;**
- Others are non-capsulated



# Microbiological diagnosis of anaerobic infections:

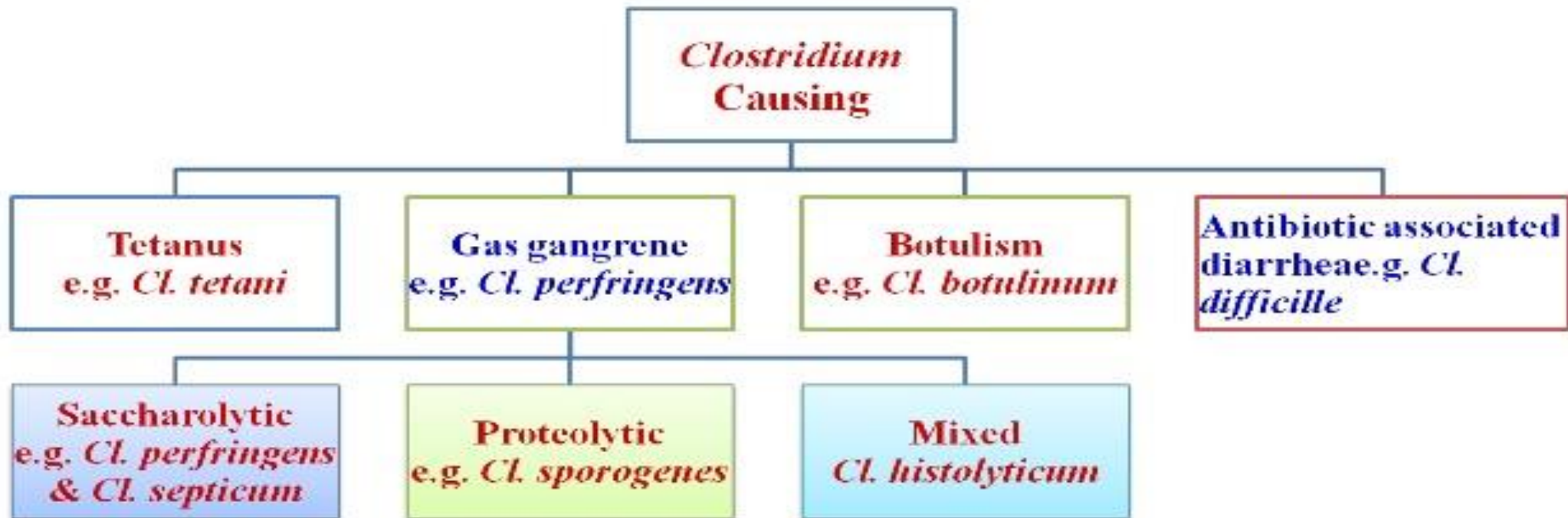
- **Examination material** - wound exudate, edema fluid, necrotic tissue, cadaver material, blood, feces, suspicious product, etc.
- **Examination methods:**
  - Microscopic
  - Bacteriological
  - Biological
  - Serological (IFR, ELISA, PHAR)
  - Molecular-genetic



## ***Species belonging to the genus Clostridium:***

- ***Clostridium tetani***
- ***C. botulinum***
- ***C. perfringens***
- *C. bifermentas*
- *C. histolyticum*
- *C. novyi*
- *C. septicum*
- ***C. difficile***

## Clostridia of medical importance

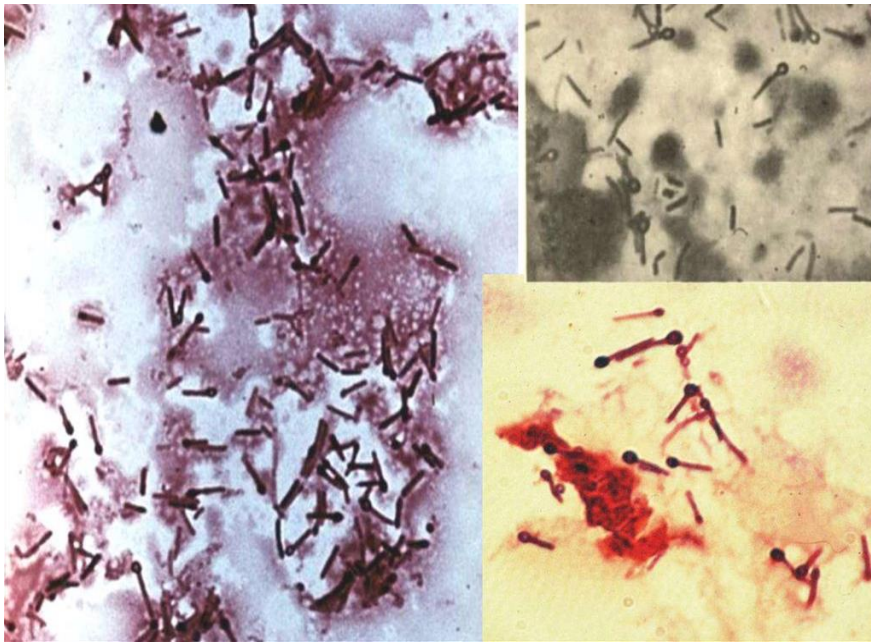




# *Clostridium tetani*

(morpho-biological characteristics)

*C. tetani* is a large Gram-positive rod-shaped bacterium measuring 4-8x0.4-1.0  $\mu\text{m}$ . It is motile, has peritrichous flagella. Inside, oval-shaped spores are located in the terminal position, giving the bacterial cell a characteristic "**drumstick**" shape.



**A smear prepared from wound exudate**



**A smear prepared from a pure culture**



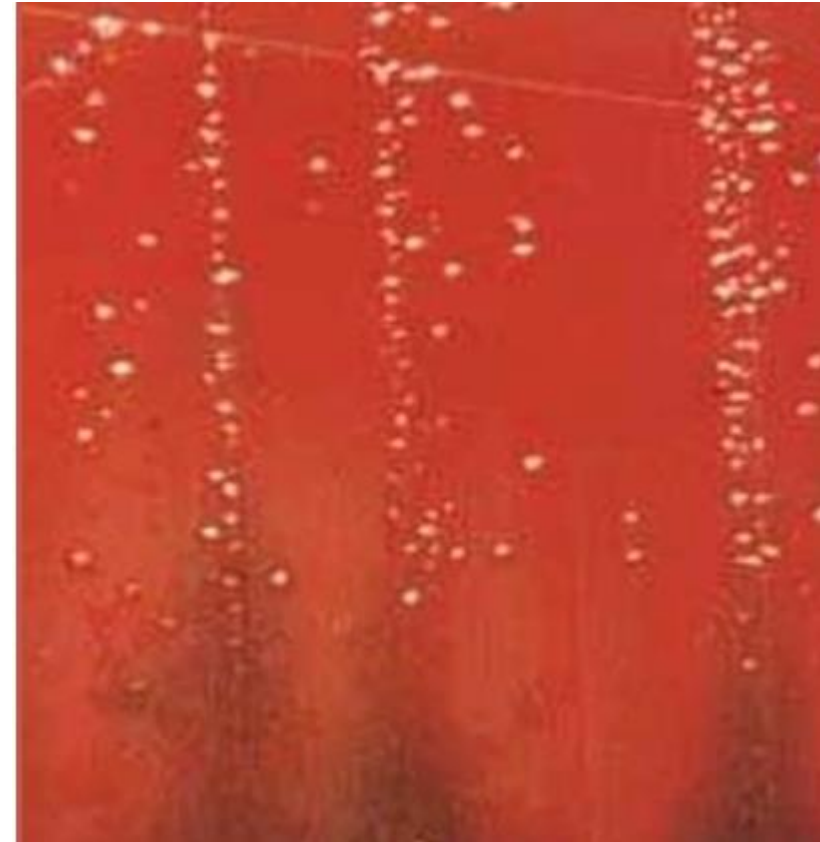
## *Clostridium tetani* (cultural characteristics)

- It is obligate anaerobic. Cultivated in nutrient media for anaerobes - Kitt-Tarotsi medium, sugar and blood agars at 35-37°C, pH 6.8-7.4.
- After 3-4 days of incubation in blood-sugar agar, it forms R-colonies with grayish, sometimes transparent, uneven granular surface and protruding edges.
- Deep in the sugar agar column, it forms cotton ball, sometimes pea-like dark colonies.
- In blood agar, a hemolysis area is observed around the colonies.
- It develops by forming turbidity in the Kitt-Tarotsi medium, darkening of the Wilson-Blair medium is observed.

***Clostridium tetani***  
Blood sugar agar colonies



***Clostridium tetani***  
**BLOOD AGAR**



***Clostridium tetani***  
***Cultural characteristics:***




***Kitt-Tarozzi medium***  
***(blurring)***



***Wilson-Blair medium***  
***(darkening)***

## *Clostridium tetani* (biochemical activity)

Species	Egg – yolk agar		Hydrolysis of gelatin	Digestion of casein	Indole production	Acid production			
	Lecithinase	Lipase				Glucose	Lactose	Sucrose	Maltose
 <i>C. tetani</i>	-	-	+	-	✓	-	-	-	-
<i>C. botulinum</i> I	-	+	+	+	-	+	-	-	+
<i>C. chauvoei</i>	-	-	+	-	-	+	+	+	+
<i>C. septicum</i>	-	-	+	+	-	+	+	-	+
<i>C. novyi</i> A	+	+	+	-	-	+	-	-	+
<i>C. perfringens</i>	+	-	+	+	-	+	+	+	+



# Methods of transmission

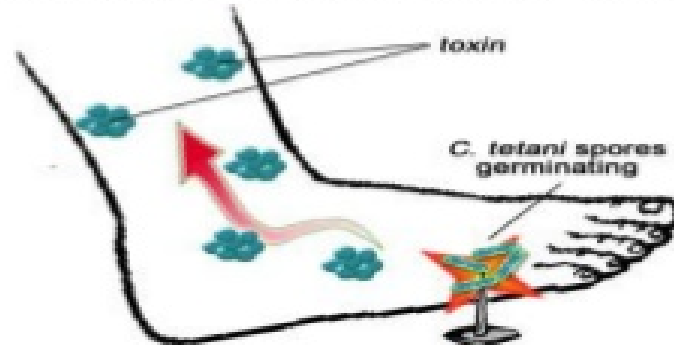
- *C. tetani* can live for years as spores in animal feces and soil. As soon as it enters the human body through a major or minor wound and the conditions are anaerobic, the spores germinate and release the toxins.
- Tetanus may follow burns, deep puncture wounds, ear or dental infections, animal bites, abortion.
- Only the growing bacteria can produce the toxin.
- It is the only vaccine-preventable disease that is *infectious but not contagious* from person to person.

# Methods of transmission

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- **It is the only vaccine-preventable disease that is *infectious but not contagious* from person to person.**

# **C.tetani - *Entry of spores***

- **Entry of *C. tetani* into the body usually involves implantation of spores into a wound**
- **After gaining entry, *C. tetani* spores can persist in the body for months, waiting for the proper low oxygen growth conditions to develop**

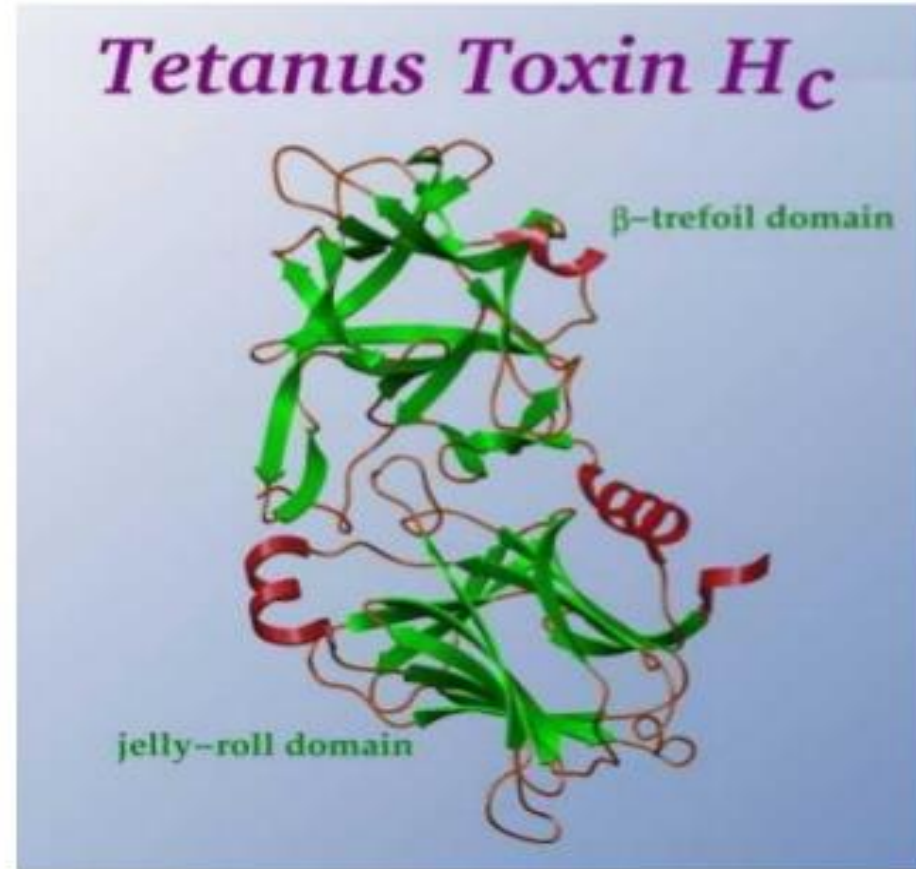


## C.tetani produces toxigenic Disease

- *C. tetani* spores enter the body, they are again in an oxygen-free environment where they can germinate. The spores usually enter the body through a deep puncture wound or cut, but animal bites or even a splinter also can allow spore entry. The bacteria then produce tetanus toxins, which circulate in the body. One of the toxins blocks nerve impulses that allow muscles to relax. This toxin is responsible for causing generalized tetanus, the most common form of the disease.

# Virulence & Pathogenicity

- Not pathogenic to humans and animals by invasive infection but by the production of a potent protein toxin
  - tetanus toxin or tetanospasmin
  - The second exotoxin produced is tetanolysin—function not known.

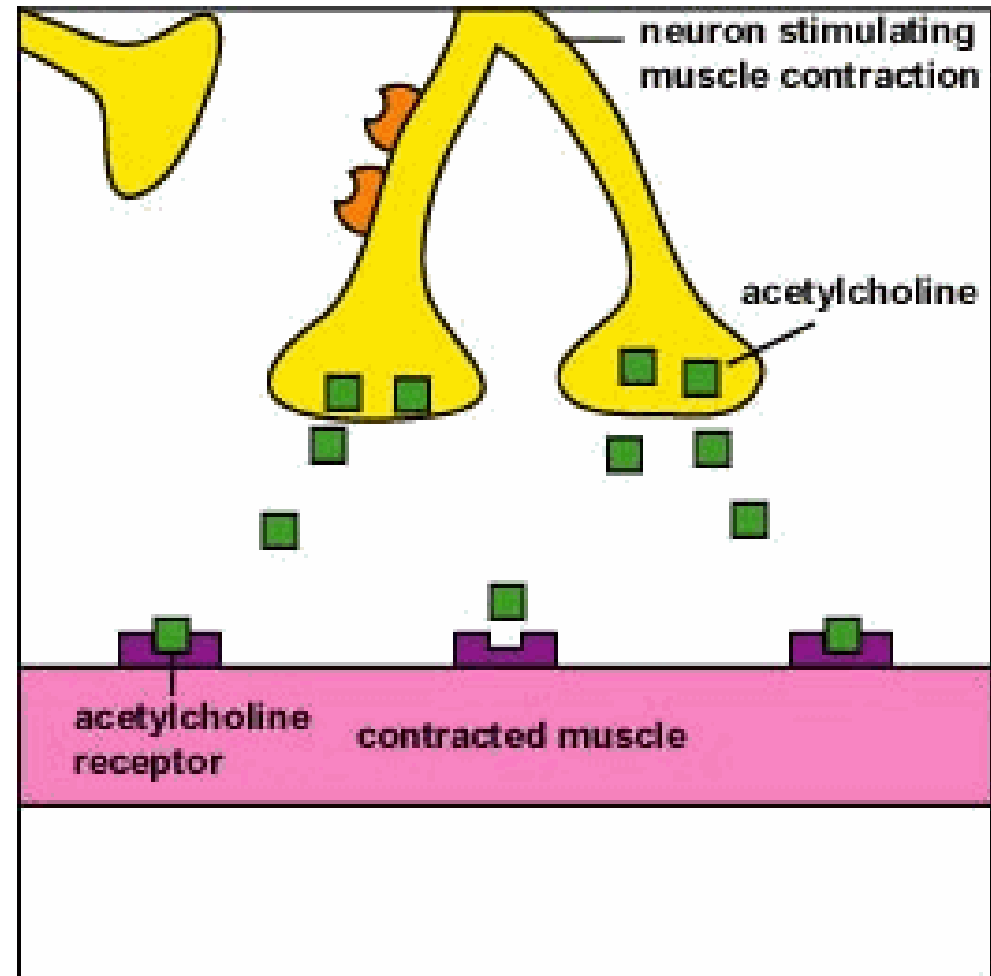
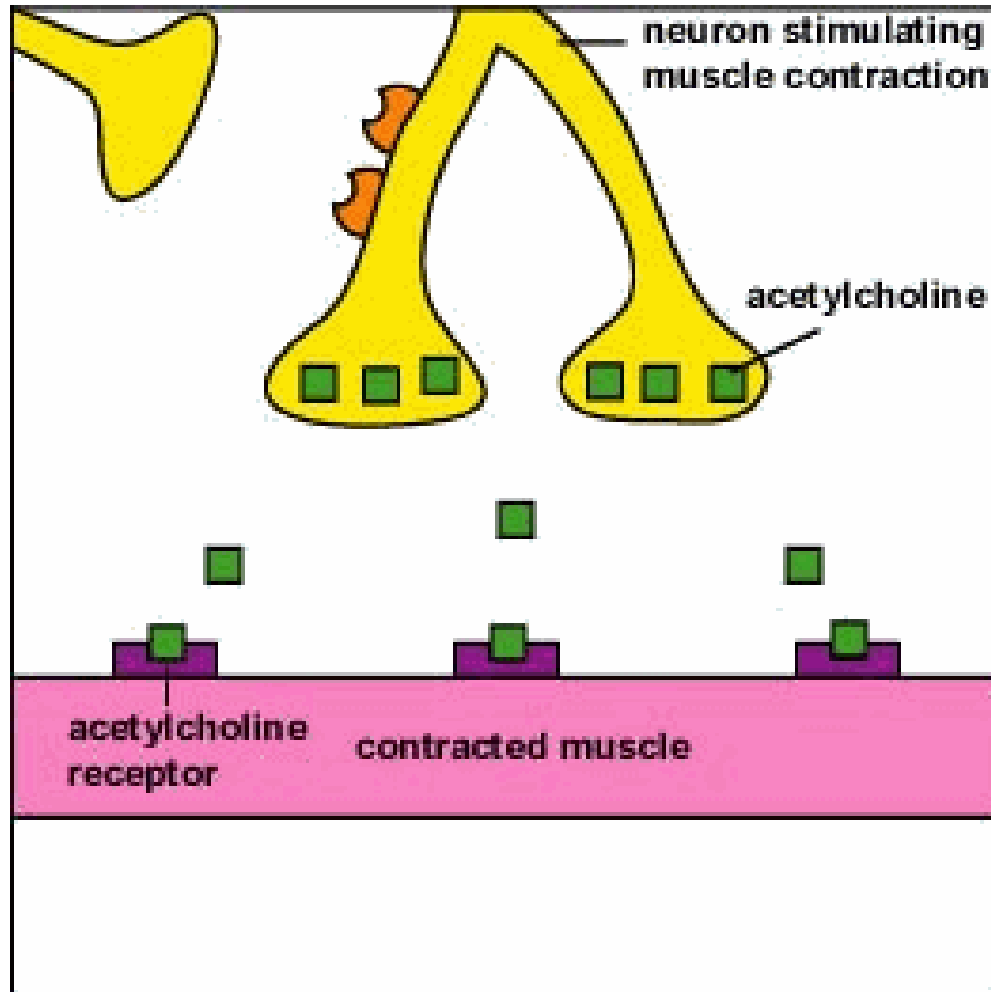




# Toxin and C.tetani

- Tetanospasmin ( exotoxin ) produced locally , released into bloodstream .
- Binds to peripheral motor neuron terminals & nerve cells of ant.horn of spinal cord
- The toxin after entering axon , transported to nerve cell body in brain stem & spinal cord – retrograde intraneuronal transport
- Toxin – migrates across synapse – presynaptic terminals- blocks the release of Glycine & GABA from vesicles.

# Mechanism of toxin



# Tetanus: Pathogenesis and clinical findings

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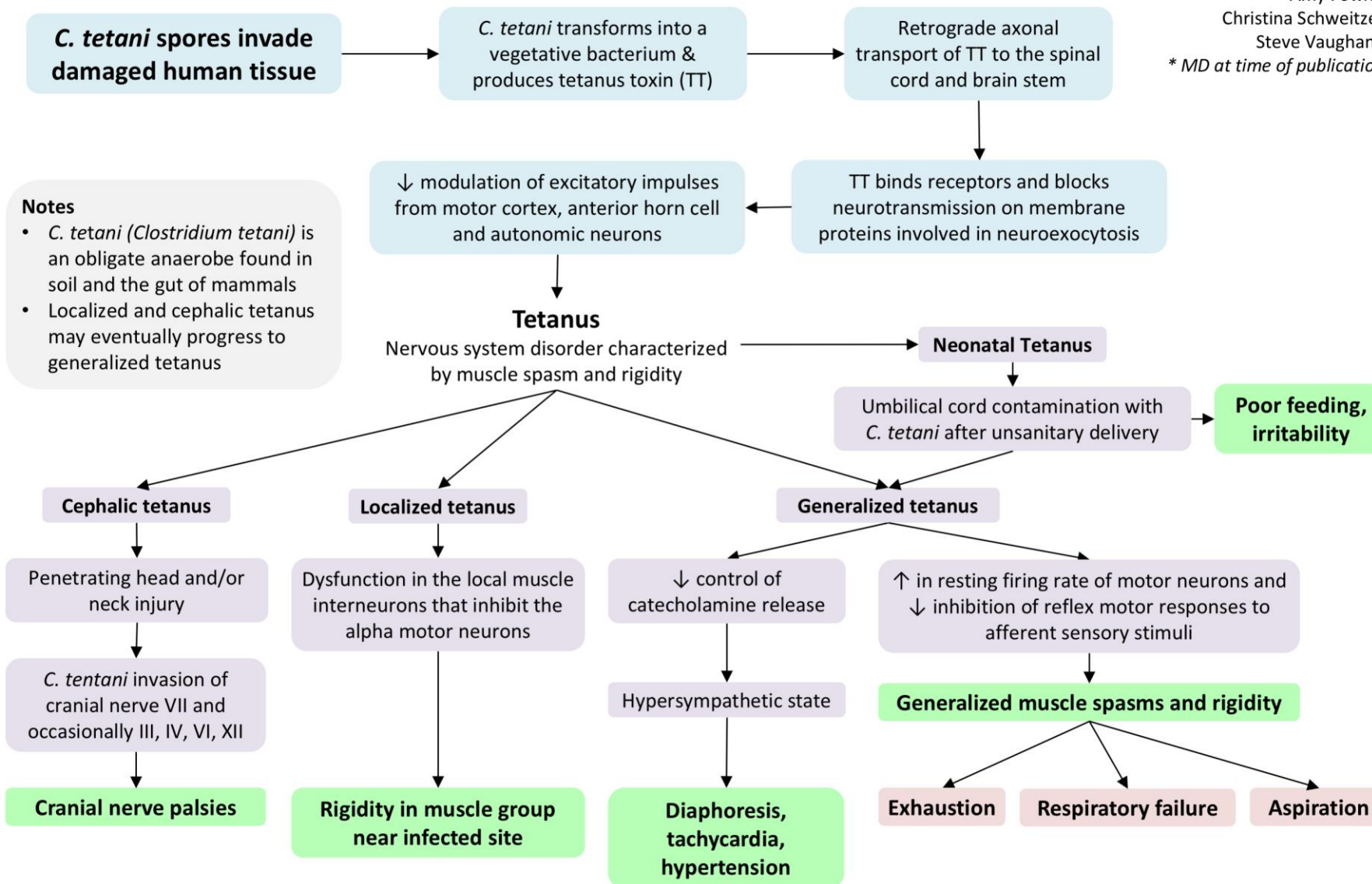
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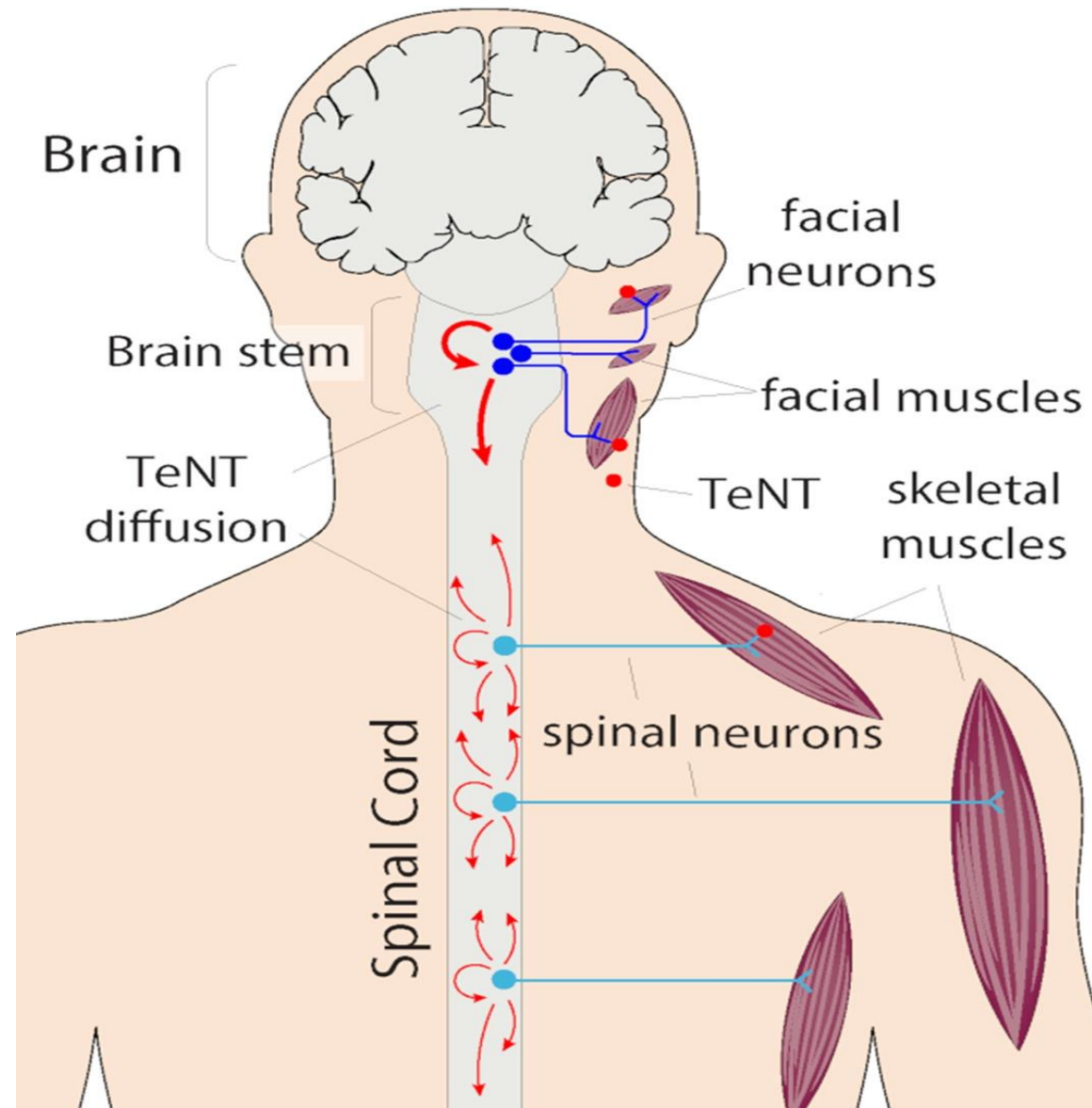
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Steve Vaughan\*

\* MD at time of publication

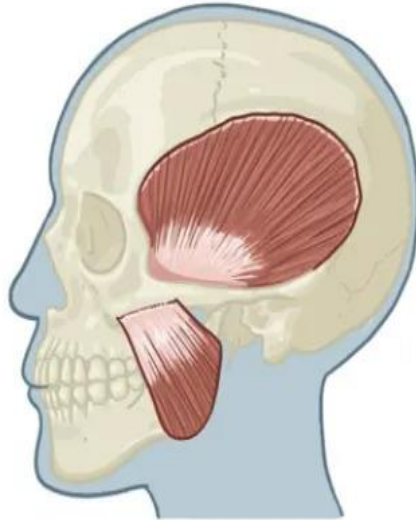


# Tetanus - Pathogenesis



# Tetanus – clinical finding

## TETANIC TRIAD



### TRISMUS "LOCKJAW"

↳ MILD → SEVERE  
SPASMS of LOWER JAW

### RISUS SARDONICUS

#### "SARDINIAN GRIN"

↳ GRIN caused by FACIAL  
MUSCLE SPASM



### OPISTHOTONOS

↳ SEVERE SIMULTANEOUS  
SPASM of ALL MUSCLES



# Symptoms

- **Tetanic seizures (painful, powerful bursts of muscle contraction)**
- if the muscle spasms affect the larynx or chest wall, they may cause asphyxiation
- stiffness of jaw (also called lockjaw)
- stiffness of abdominal and back muscles
- contraction of facial muscles
- fast pulse
- fever
- sweating



# **Patient Manifests with**

- **A person suffering from tetanus undergoes convulsive muscle contractions of the jaw--called LOCKJAW**
- **The contractions by the muscles of the back and extremities may become so violent and strong that bone fractures may occur**
- **The affected individual is conscious throughout the illness, but cannot stop these contractions**

# Trismus







***The back muscles are more powerful, thus creating the arc backward***

***"Opisthotonus" by Sir Charles Bell, 1809.***



***Baby has neonatal tetanus with complete rigidity***

## **Types of tetanus:** **local, cephalic, generalized, neonatal**

- *Incubation period: 3-21 days, average 8 days.*

### **Uncommon types:**

- **Local tetanus:** persistent muscle contractions in the same anatomic area as the injury, which will however subside after many weeks; very rarely fatal; milder than generalized tetanus, although it could precede it.
- **Cephalic tetanus:** occurs with ear infections or following injuries of the head; facial muscles contractions.



# Most common types:

## Generalized tetanus

- descending pattern: lockjaw → stiffness of neck → difficulty swallowing → rigidity of abdominal and back muscles.
- Spasms continue for 3-4 weeks, and recovery can last for months
- Death occurs when spasms interfere with respiration.

## Neonatal tetanus:

- **Form of generalized tetanus that occurs in newborn infants born without protective passive immunity because the mother is not immune.**
- **Usually occurs through infection of the unhealed umbilical stump, particularly when the stump is cut with an unsterile instrument.**

## Methods of diagnosis

- Based on the patient's account and physical findings that are characteristic of the disease.
- Diagnostic studies generally are of little value, as cultures of the wound site are negative for *C. tetani* two-thirds of the time.
  - When the culture is positive, it confirms the diagnosis of tetanus

# Diagnosis

- Tests that may be performed include the following:
  - Culture of the wound site (may be negative even if tetanus is present)
  - Tetanus antibody test
  - Other tests may be used to rule out meningitis, rabies, strychnine poisoning, or other diseases with similar symptoms.



# Clinical treatment

- If treatment is not sought early, the disease is often fatal.
- The **bacteria** are killed with antibiotics, such as penicillin or tetracycline; further toxin production is thus prevented.
- **The toxin is neutralized with shots of tetanus immune globulin, TIG.**
- Other drugs may be given to provide sedation, relax the muscles and relieve pain.
- Due to the extreme potency of the toxin, immunity does not result after the disease.

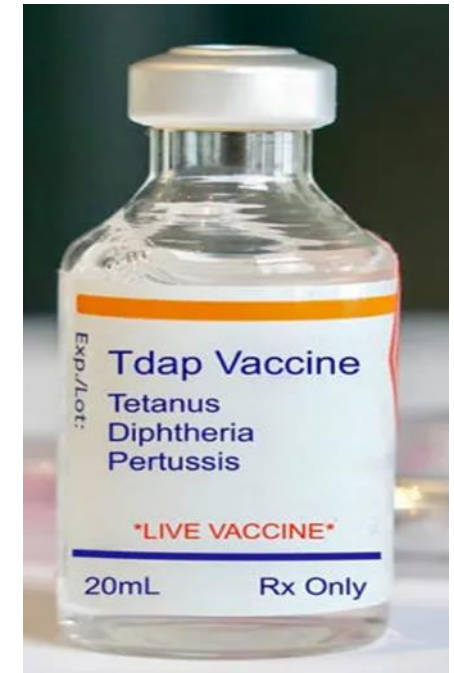


## Method of prevention - Immunization

- A person recovering from tetanus should begin active immunization with tetanus toxoid (Td) during convalescence.
- The tetanus toxoid is a formalin-inactivated toxin, with an efficiency of approx. 100%.
- The **DTaPe** includes tetanus, diphtheria and pertussis toxoids; it is routinely given in the US during childhood. After 7 years of age, only Td needs to be administered.
- Because the antitoxin levels decrease over time, booster immunization shots are needed every 10 years.

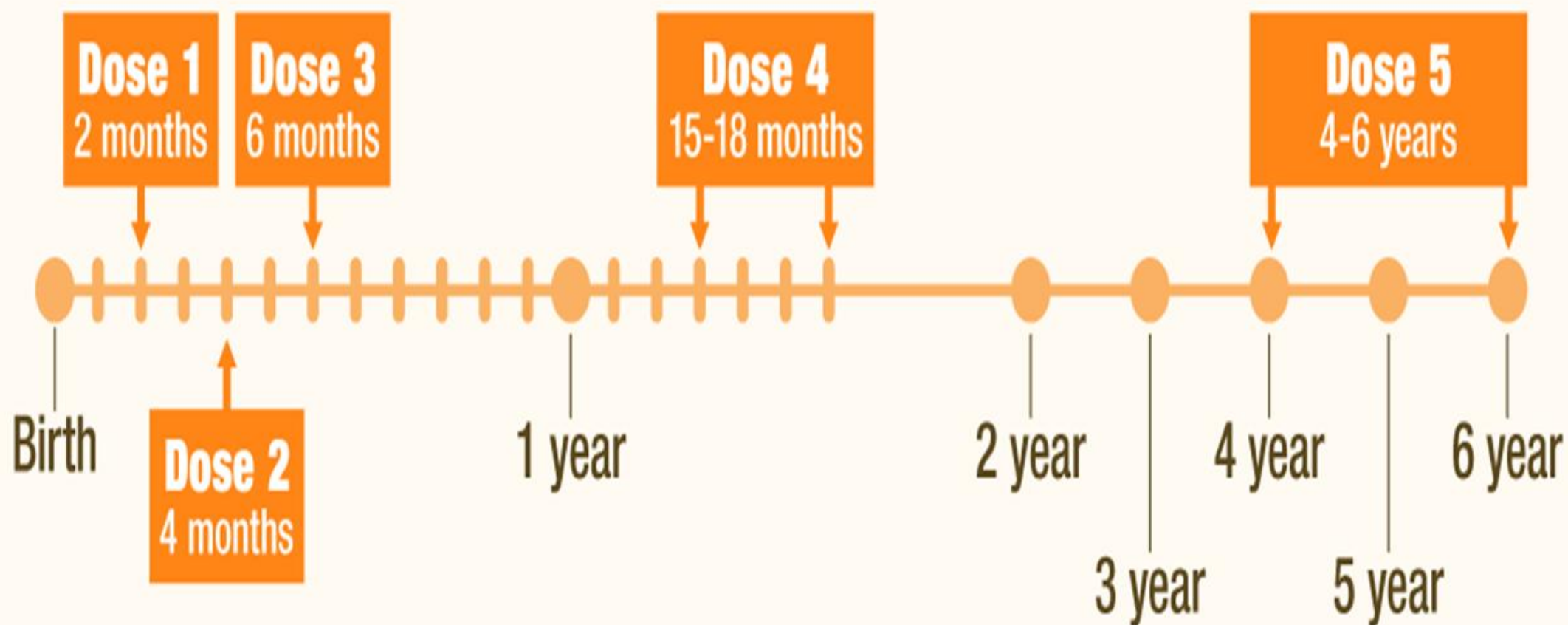
Dr.T.V.Rao MD

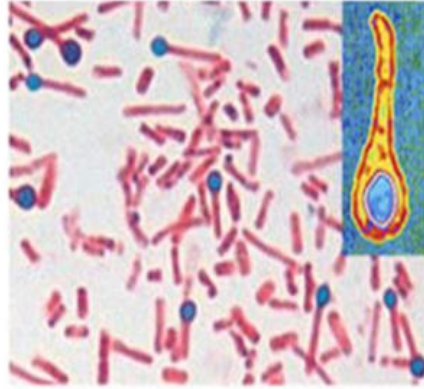
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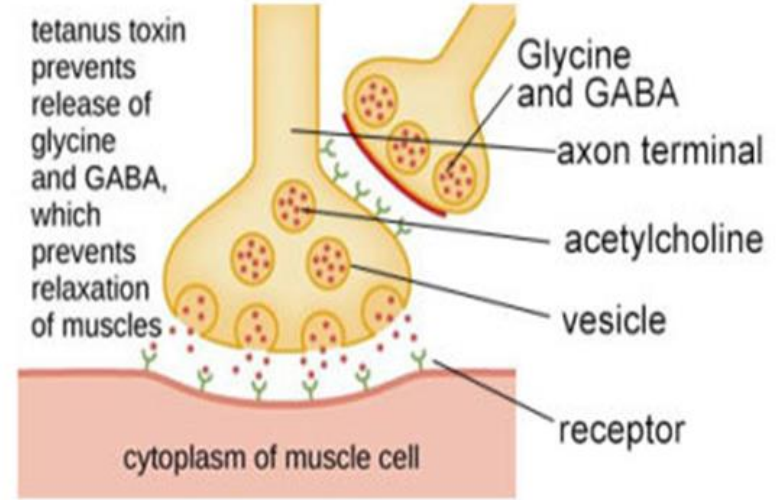


# DTaP: Diphtheria, Tetanus, and Acellular Pertussis





'Drum Stick' or  
'Tennis Racket' appearance



Mechanism of Tetanus Toxin

# ***Clostridium tetani***



Opisthonus

microbeonline



Risus Sardonicus



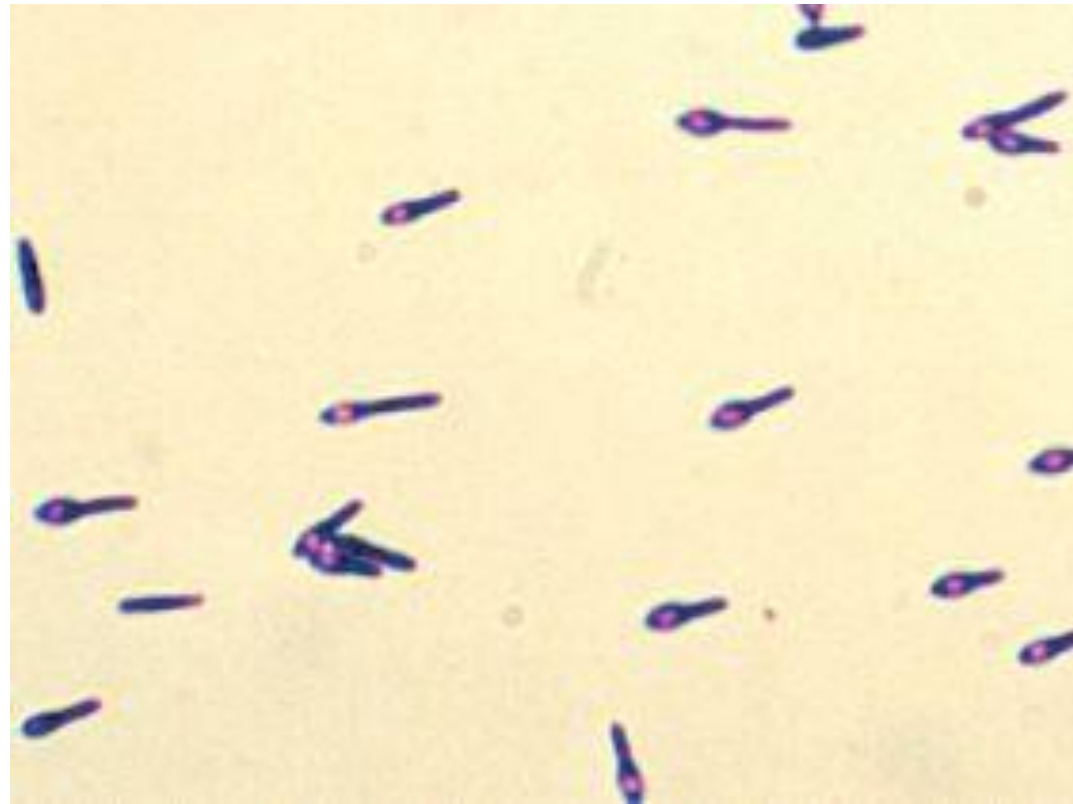
## *C.botulinum*

### **Classification:**

- 1.Domain –bacteria
2. Division –firmicutes
3. Class –clostridia
4. Order –clostridiales
5. Family –closridiacae
6. Genus –clostridium
7. Species -botuulinium

## *Clostridium botulinum* (morpho-biological characteristics)

- *C.botulinum* is a 4-9x0.6-1.0  $\mu\text{m}$  size polymorphic rod-shaped bacterium. It is motile, has peritrichous flagella. It does not form a capsule, but under unfavorable conditions produces subterminal spores (similar to a tennis racket).



*C.botulinum*

## *Clostridium botulinum* (cultural characteristics)

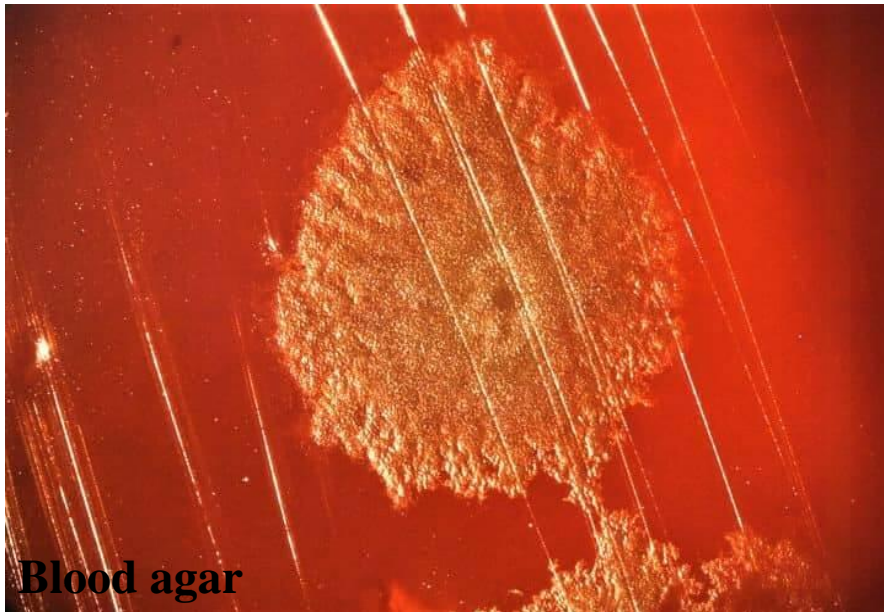
- It is obligate anaerobic. Cultivated at 25-37°C pH 7.3-7.6.
- On blood-sugar agar, colonies surrounded by a hemolysis zone with irregularly shaped, thread-like protrusions, on blood agar, they form smooth (S-shaped) with a bright surface similar to a dew drop, or R-colonies with indented-protruding edges.
- In the depth of the sugar agar column, pea-like (S-shaped), sometimes cotton ball-like (R-shaped) colonies are formed.
- It produces turbidity and gas in liquid media (Kitt-Tarozzi medium, liver broth, etc.).



# *Clostridium botulinum* (cultural characteristics)



**Egg yolk agar**



**Blood agar**



***Kitt-Tarozzi medium***  
(blurring)

## Clostridium botulinum (biochemical activity)

Species	Egg – yolk agar		Hydrolysis of gelatin	Digestion of casein	Indole production	Acid production			
	Lecithinase	Lipase				Glucose	Lactose	Sucrose	Maltose
<i>C. tetani</i>	-	-	+	-	✓	-	-	-	-
<i>C. botulinum</i> I	-	+	+	+	-	+	-	-	+
<i>C. chauvoei</i>	-	-	+	-	-	+	+	+	+
<i>C. septicum</i>	-	-	+	+	-	+	+	-	+
<i>C. novyi</i> A	+	+	+	-	-	+	-	-	+
<i>C. perfringens</i>	+	-	+	+	-	+	+	+	+



## TOXIN

- **Virulence factor**—botulinum toxin
  - neurotoxin
  - relatively heat-labile and resistant to protease
  - types: A, B, C, D, E, F, G
  - the most potent toxic material known

10,000 times

□ mechanism of action

Toxin → gut → blood → cholinergic synapses → block the release of exciting neurotransmitter, e.g., acetylcholine → flaccid paralysis

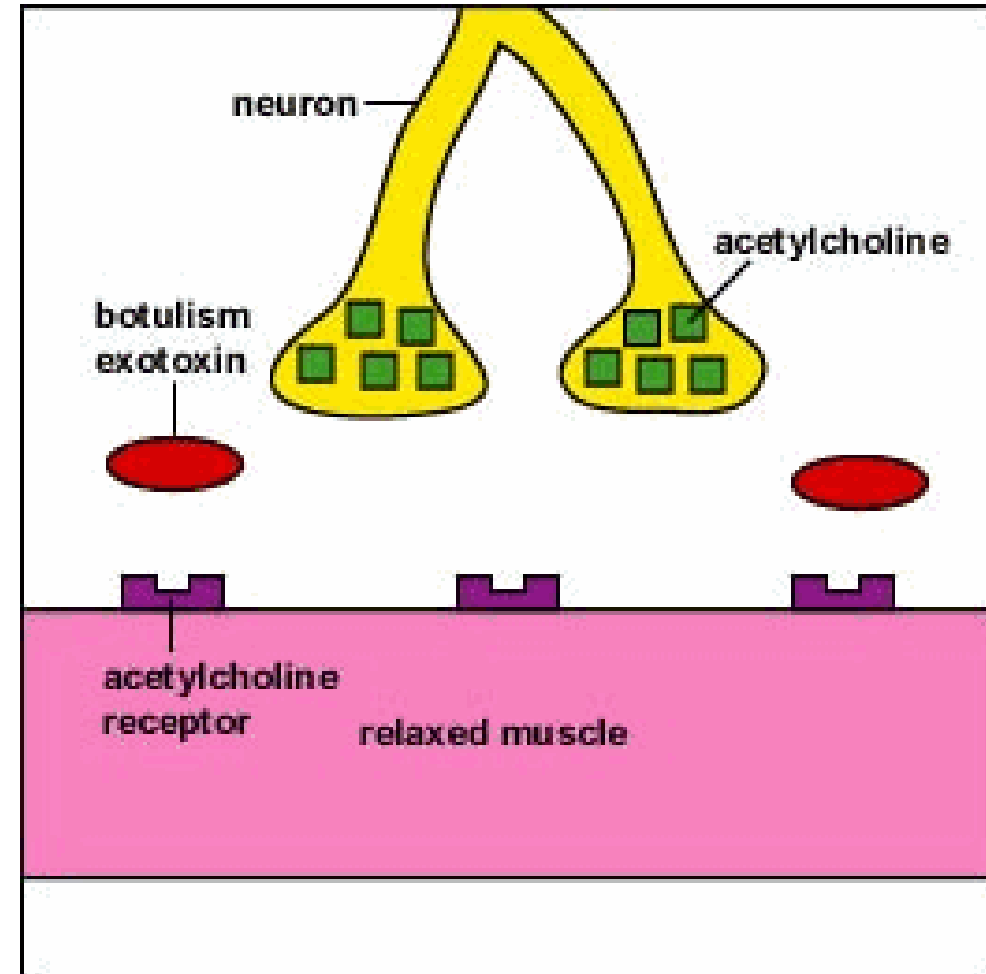
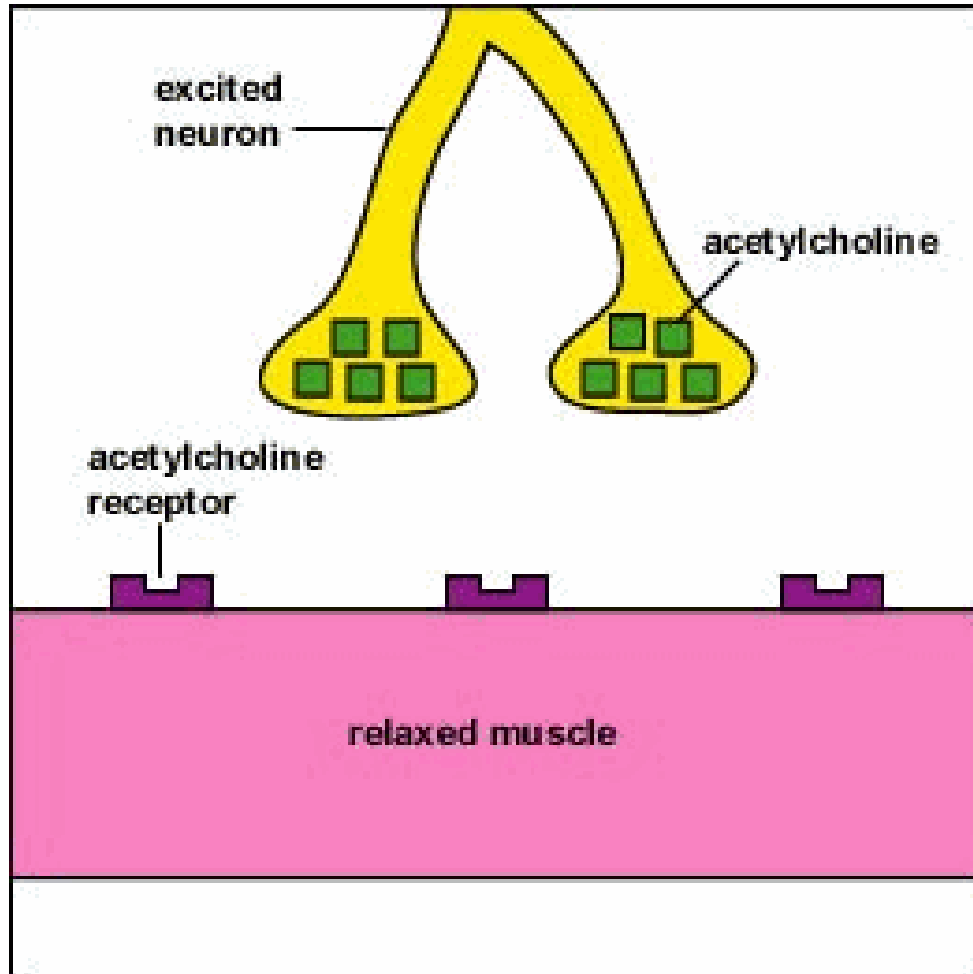


## MECHANISM OF TOXIN.

- **Botulinum toxin:**

- absorbed from the gut
- Binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves.
- Proteolysis-by the light chain of botulinum toxin of the target SNARE proteins in the neurons inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and paralysis
- SNARE proteins are-synaptobrevin, SNAP 25, syntaxin.
- Type A and E toxin cleaves-SNAP 25
- Type B toxin cleaves synaptobrevin

# Mechanism of toxin



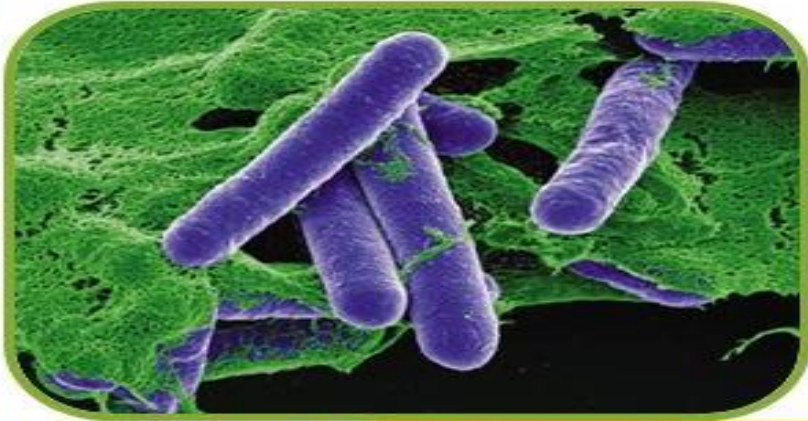
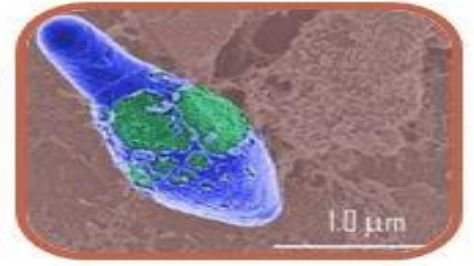


**Canned meat, fish and vegetable products can cause botulism!**



# Botulism

- Latin: *botulus* = sausage
- Is a rare but serious paralytic illness caused by botulinum toxin, which is produced by the bacterium *Clostridium botulinum* under anaerobic conditions



*Clostridium botulinum*

-Gram-positive Bacillus

-Spores

-Found in soil, vegetables, fruits  
and animal feces

# Pathogenesis

- **Transmitted in three ways:**
  - Food or water toxin contamination.
  - Wound infected with *C. Botulinum*.
  - Ingestion of *C. botulinum*.
- **Most common contaminated food: Vacuum packed, or canned alkaline food.**  
**E.g. fish, green beans, any home-canned food.**
- **Foods eaten without cooking.**

# Pathogenesis

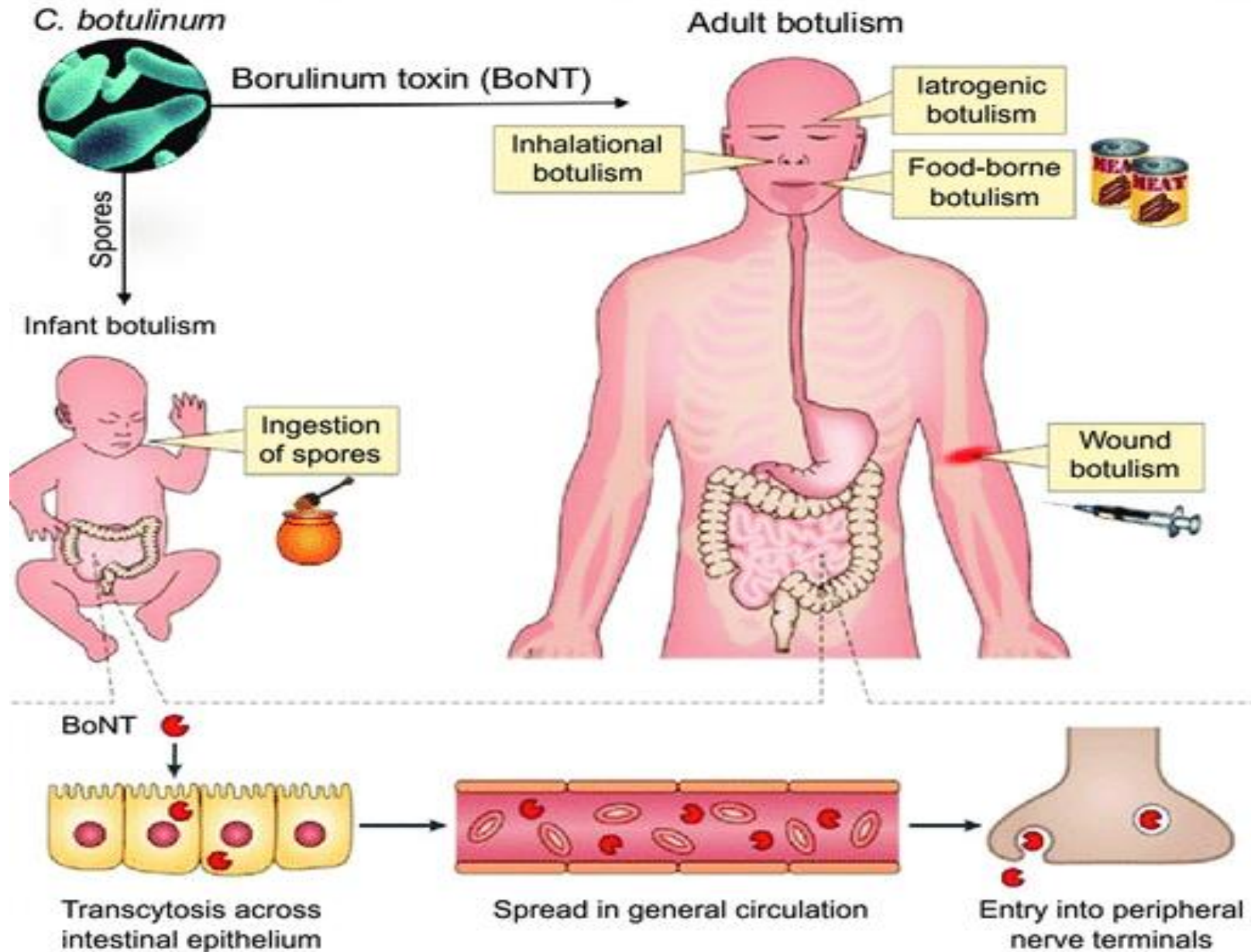
- Toxin must enter body
  - Direct toxin absorption from mucosal surface
    - Gut – foodborne
    - Lungs – inhalational
  - Via toxin produced by infection with *C.botulinum*
    - Skin breaks – wound botulism after trauma, IV drugs
    - Gut – intestinal botulism
    - Would not be seen in BT event, as toxin would be used
- Does not penetrate intact skin



# Pathogenesis of Infant Botulism

- Cause :infection by C. botulinum.
- Age: 5 - 20 weeks of age.
- Characterized by constipation and weak sucking.
- It cause “**sudden infant death syndrome-SIDS**”.
- found in the stool.

# Botulism – pathogenesis



## CLINICAL MANIFESTATIONS

- **Adult botulism:**

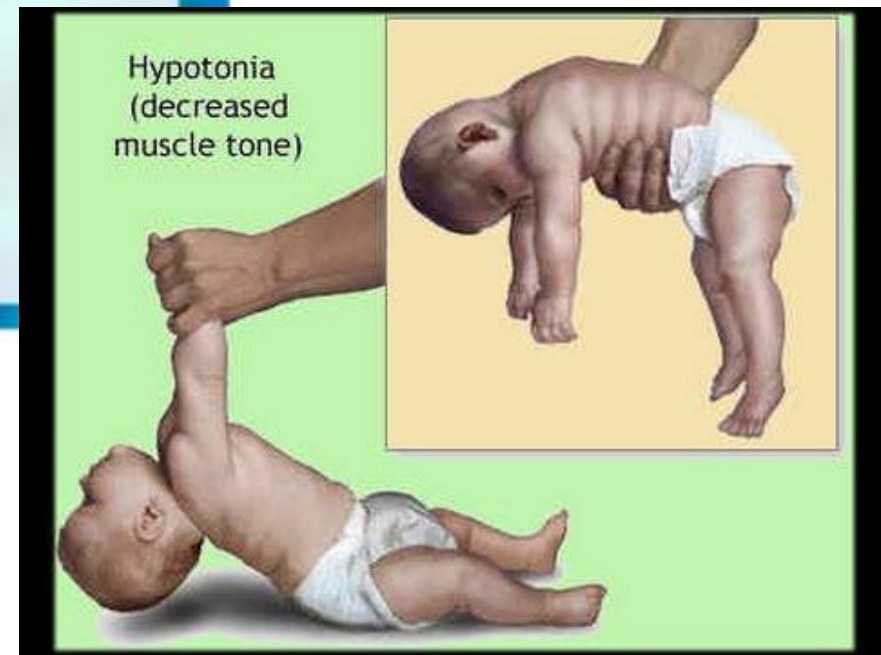
flaccid paralysis: double vision, dysphagia, difficulty in breathing & speaking ,rare gastrointestinal symptoms .cause of death: respiratory failure

- **Infant botulism:**

- manifestation: constipation, poor feeding, difficulty in sucking and swallowing, weak cry, loss of head control.

### **Floppy baby**

prevention: free of honey



# Symptoms

- **Appear in 18-24 hrs.**
- **include :**
  - Blurry vision, Double vision.**
  - Dry mouth.**
  - Trouble swallowing.**
  - Trouble breathing.**
  - Muscle weakness.**

**Fever is not a symptom of botulism**

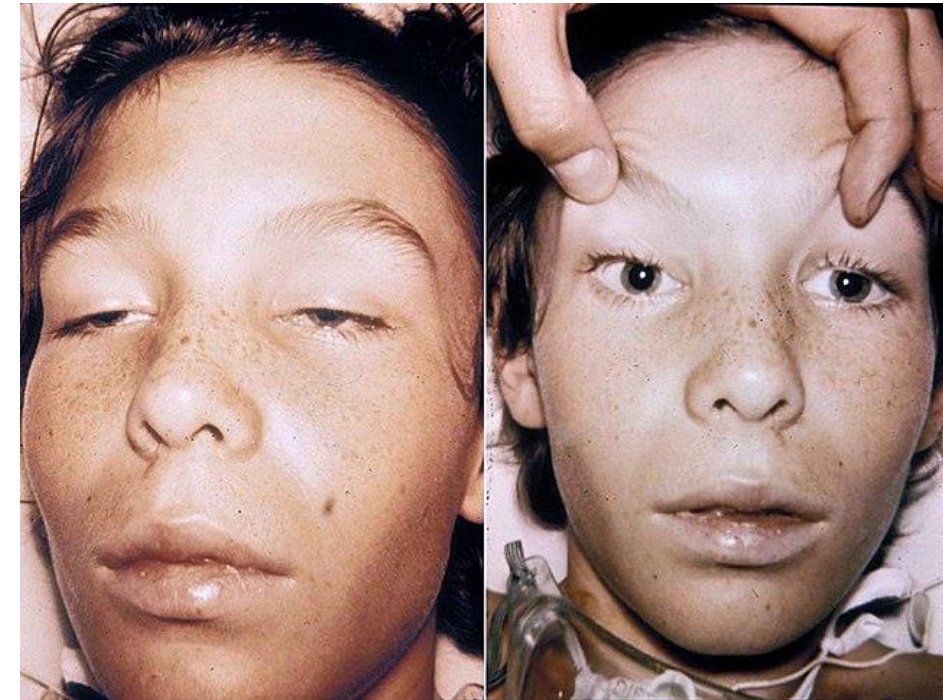


# Clinical Features

- Classic Triad
  - Symmetric, descending flaccid paralysis with prominent bulbar palsies
  - Afebrile
  - Clear sensorium
- Bulbar palsies summarized as "4 Ds"
  - Diplopia, dysarthria, dysphonia, dysphagia

## Botulism symptoms: Characteristic Triad

- **Symmetric, descending (cranial nerves first, then upper extremities, then respiratory muscles, and lower extremities) flaccid paralysis with prominent bulbar palsies, particularly:**
  - **Diplopia** – double vision
  - **Dysarthria** – difficulty in speech articulation
  - **Dysphonia** – difficulty in voice production
  - **Dysphagia** – difficulty in swallowing
- **Patient is afebrile (although fever may be present in wound botulism)**
- **Patient's sensibilities intact; cognitive functions unaffected**



# Diagnosis

- **Clinical diagnosis**
- Diagnostic tests help confirm
  - Toxin neutralization mouse bioassay
    - Serum, stool, or suspect foods
  - Infant botulism
    - *C botulinum* organism or toxin in feces
    - Testing is done in Reference laboratories under Biosafety regulations

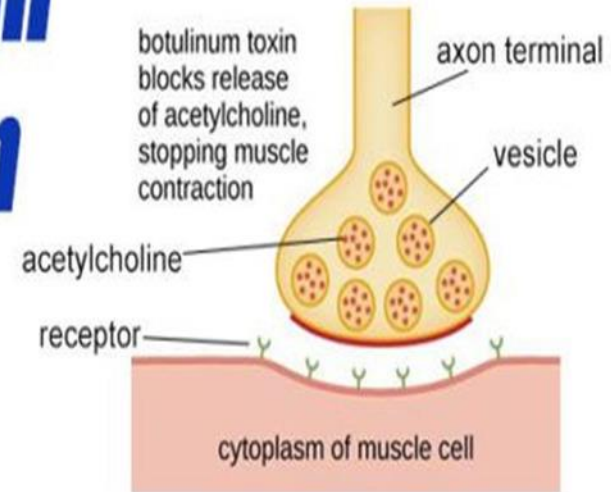




Gram-positive bacilli  
with 'Tennis Racket' appearance

# ***Clostridium botulinum***

microbeonline

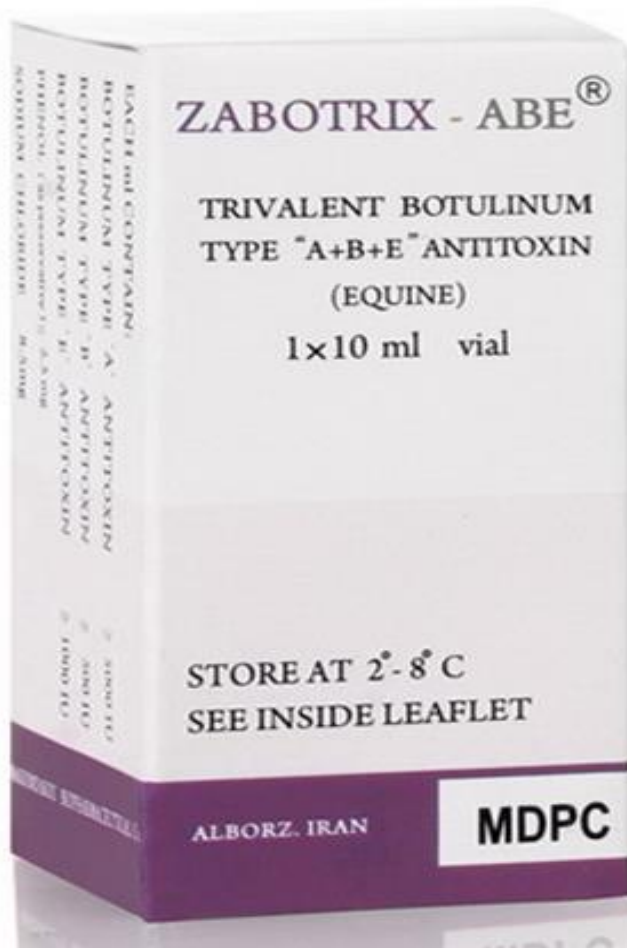


Mechanism of Botulinum Toxin



# Treatment

- Trivalent (A,B,E) antitoxin must administered intravenously (recovery takes several weeks)
- Mechanical respirator is administered if necessary.



# Treatment

- **Antitoxin action**

- Food-borne botulism
  - Neutralizing antibody levels exceed toxin levels
  - Single dose adequate
- Large exposure (e.g. biological weapon)
  - can confirm adequacy of neutralization
    - recheck toxin levels after treatment

- **Antitoxin adverse effects**

- Serum sickness (2-9%), anaphylaxis (2%)



## Causes of gas gangrene:

<b>C.perfringens (90-100%)</b>	<b>C.septicum (4-12%)</b>
<b>C.novyi (25-30%)</b>	<b>(spontan)</b>
<b>C.histolyticum (0,1 %)</b>	<b>C.bifermentas</b>
<b>C.sordellii</b>	<b>C.sporogenes</b>

# Clostridium perfringens

- Large rectangular Gram positive bacillus
- Spores seldom seen in vivo or in vitro
- non motile
- Produces several toxins
  - **alpha (lecithinase), beta, epsilon .....**
  - **enterotoxin**
- Causes a spectrum of human diseases
  - **Bacteraemia**
  - **Myonecrosis**
  - **food poisoning**
  - **enteritis necrotica (pig bel)**



# **1. *Clostridium perfringens* (Cl. welchii)**

## **Morphology**

- Large Gram-positive bacilli with straight, parallel sides & slightly rounded ends.
- Measure 4-6x1µm in size, occurring singly or in chains
- Pleomorphic, capsulated & non-motile.
- Spores are **central or sub terminal**. **Spores are rarely seen in culture media or material from pathogenic lesions**, a characteristic morphologic feature



## CULTURAL CHARACTERISTICS

- Robertson's cooked meat broth** is ideal; meat is turned **pink but not digested** with sour odor.
- Stormy fermentation** of lactose in litmus milk; the acid coagulates casein-**acid clot**.
- On BAM**: Target haemolysis

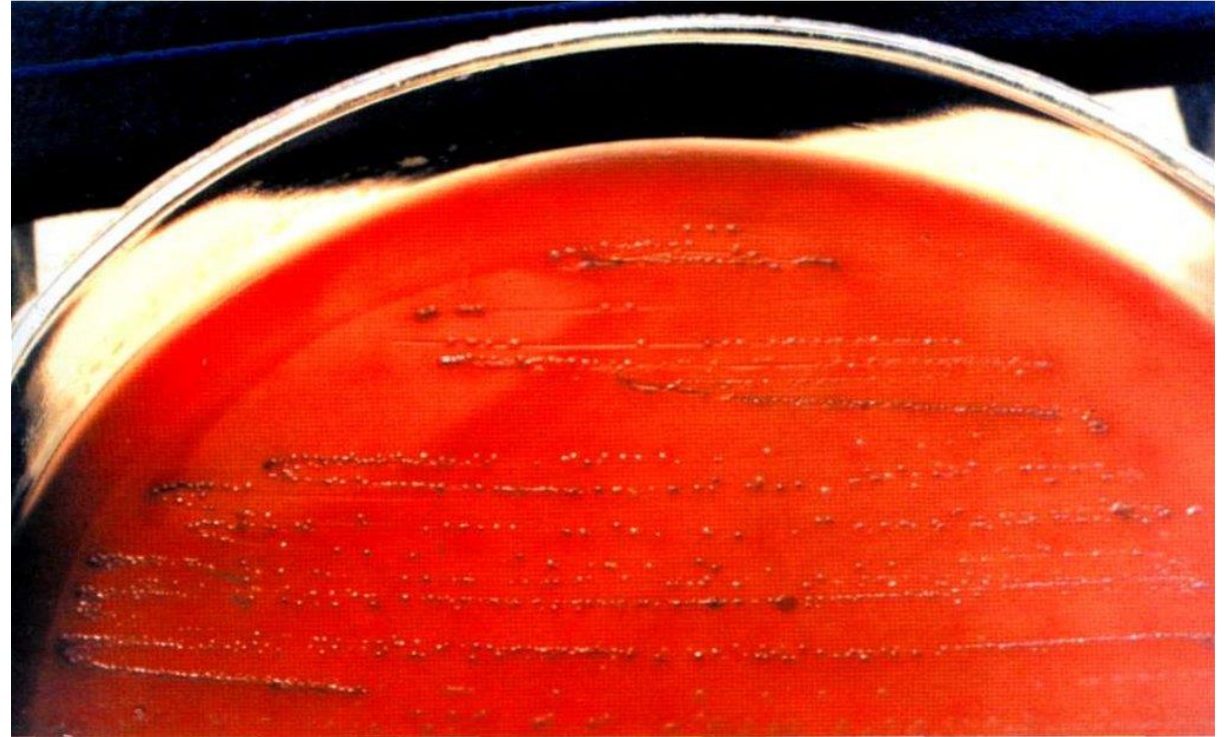
## BIOCHEMICAL REACTIONS:

Glucose	} Fermented with A & G production	Indole	-ve
Lactose		MR	+ve
Maltose		VP	-ve
-H <sub>2</sub> S prodn. test & Nitrate redn. test -			+ve

## *Clostridium perfringens* (cultural characteristics)



*Blood agar*



*Sessler agar*



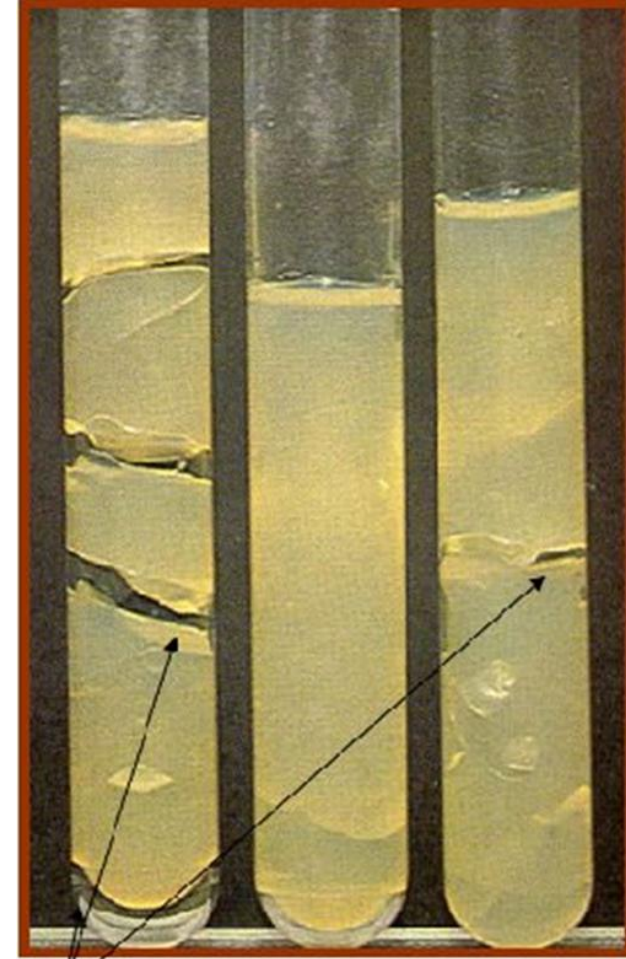
## *Clostridium perfringens* (cultural characteristics)



*Kitt-Tarozzi medium*



*Wilson-Blair medium*



Sugar agar due to gas formation  
agar column digestion



**RESISTANCE:** Boiling for 5 min. & autoclaving at 121°C for 15 minutes kills all spores.

**TOXINS:** 12 distinct types of toxins elaborated;

- **α toxin**-lethal, dermonecrotic & hemolytic
- **β, ε & ι toxins**- lethal & necrotizing
- **γ & η toxins**- minor lethal action
- **δ toxin**-lethal & hemolytic for red cells
- **θ toxin**-lethal & cytolytic
- **κ toxin**- collagenase
- **λ toxin**-proteinase & gelatinase
- **μ toxin**- hyaluronidase & **ν toxin**-deoxyribonuclease

## ENZYMES

- Neuraminidase**-destroys myxovirus receptors on red cells
- Hemagglutinin**-active against red cells of humans
- Fibrinolysin**
- Hemolysin**
- Bursting factor**- has specific action on muscle tissue & responsible for muscle lesions in gas gangrene.
- Circulatory factor**-causes an increase in adrenaline sensitivity of capillary bed ,also inhibits phagocytosis

TABLE 29-2

**Virulence factors of *Clostridium perfringens***

Virulence factors	Biological functions
$\alpha$ (Alpha) toxin	Lethal, dermonecrotic, hemolytic, and is a lecithinase; causes toxemia, increases vascular permeability of blood vessels, leading to tissue destruction. Main cause of toxaemia associated with gas gangrene
$\beta$ (Beta) toxin	Lethal toxin; causes necrotic lesions in necrotizing enteritis
$\epsilon$ (Epsilon) toxin	Lethal protoxin; increases vascular permeability of the wall of the gastrointestinal tract
$\iota$ (Iota) toxin	Lethal toxin; causes necrotic lesions and increases vascular permeability
$\delta$ (Delta) toxin	Hemolytic
$\theta$ (Theta) toxin	Oxygen-labile hemolytic and cytolytic toxin
$\kappa$ (Kappa) toxin	Collagenase
$\lambda$ (Lambda) toxin	Proteinase and gelatinase
$\mu$ (Mu) toxin	Hyaluronidase
$\nu$ (Nu) toxin	Deoxyribonuclease
Enterotoxin	Enterotoxic and hemolytic; alters permeability of the gastrointestinal membrane
Neuraminidase	Alters cell surface ganglioside receptors and promotes capillary permeability
Bursting factor	Causes typical muscle lesions in gas gangrene
Circulating factor	Increases adrenaline sensitivity of the capillary membrane
Hyaluronidase	Breaks down intercellular cement substance and promotes the spread of infection along tissue planes

## PATHOGENICITY

-Three Clinical conditions produced include;

**1.Simple wound contamination:** Slow wound healing

**2.Anaerobic or clostridial cellulitis:**

-Clostridia invade fascial planes(fasciitis) with minimal toxin production but no invasion of muscle tissue.

-Lesions vary from limited '**gas abscess**' to extensive involvement of limbs.

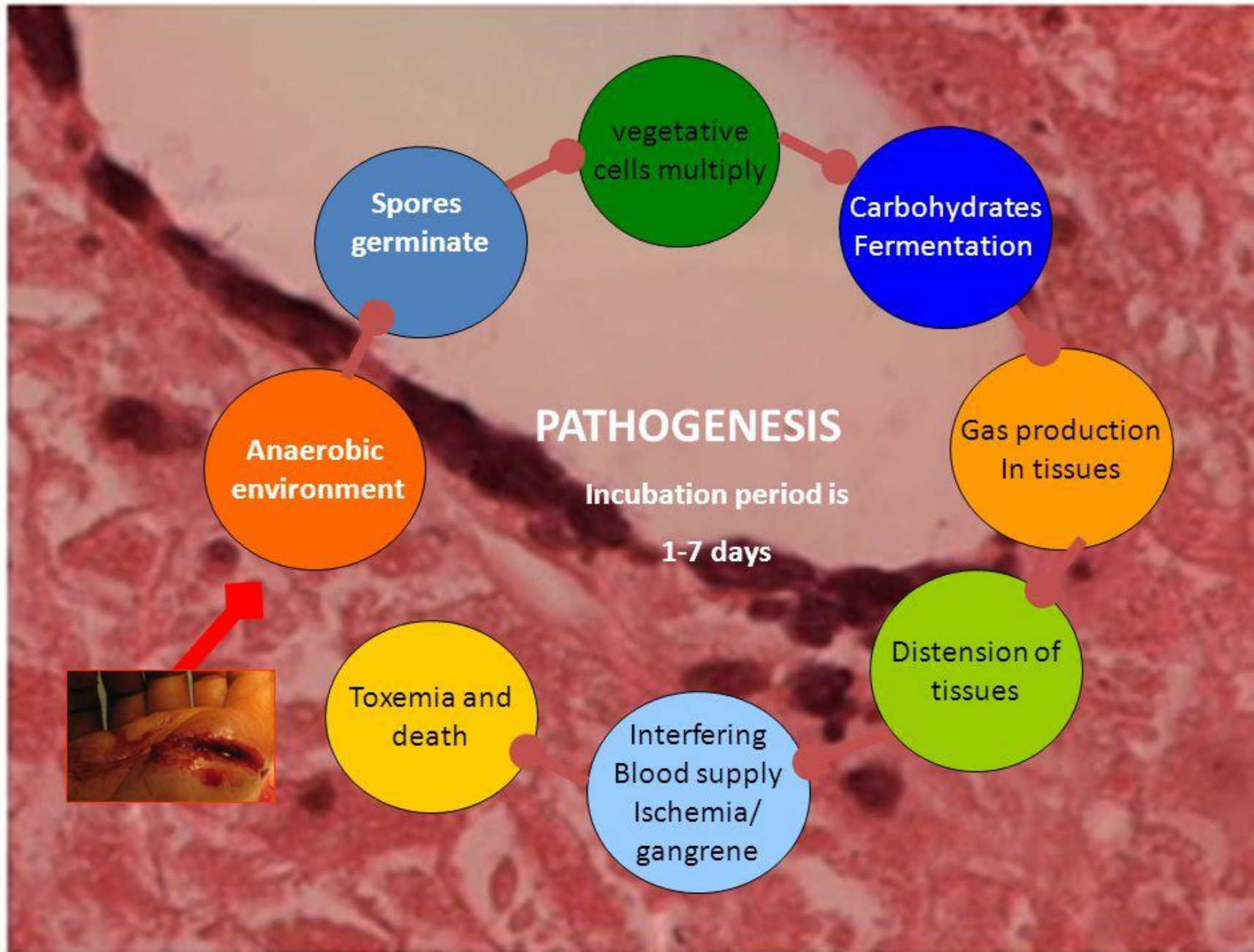
-Seropurulent discharges with offensive odor produced





### **3.Anaerobic myositis or myonecrosis or gas gangrene**

- Most serious complication of clostridial invasion of healthy muscle tissue .
- Abundant formation of exotoxin & production of gas.
- GG is disease of war. In civilian life it follows road accidents or injuries with crushing of muscle mass.
- GG is rarely infection of single clostridium; several species found in association with anaerobic streptococci & facultative anaerobes (*E.coli,Stap,Proteus*)
- Among pathogenic clostridia,*Cl.perfringens* is most frequently encountered(60%) followed by *Cl.Novyi* & *Cl.septicum*(20-40%).



## Gas gangrene:





## **OTHER INFECTIONS:**

- Food poisoning:** usually caused by **Type A** strains
- Gangrenous appendicitis:** *Cl.perfringens* **Type A**  
& occasionally by **Type D**
- Necrotizing enteritis:** caused by **Type C** strains
- Biliary tract infection:** Rare but serious -EC & PCS
- Endogenous gas gangrene of intra-abdominal origin**
- Brain abscess & meningitis:** Rare
- Panophthalmitis:** Rare
- Thoracic infections**
- Urogenital infections-** usually follow UT surgery



## Necrotizing Enteritis

- Rare, acute necrotizing process in the jejunum
- Abdominal pain, bloody diarrhea, shock, and peritonitis
- Mortality: 50%
- Beta-toxin-producing *C. perfringens* type C

## Septicemia

## Other causative agents of gas gangrene:

- *Clostridium novyi* -
- *Clostridium septicum* -
- *Clostridium histolyticum* -
- *Clostridium sordellii* -

# *Clostridium novyi*

## *morpho-biological characteristics:*

- Gram-positive is a large, straight or slightly bent rod-shaped bacterium measuring 4-22x1-2  $\mu\text{m}$ .
- Unlike *C.perfringens*, it is motile, has peritrix flagella, does not form a capsule.
- Spores are located in the subterminal position.



C.novyi

# *Clostridium novyi*

## *cultural characteristics:*

- Obligate anaerobic, very sensitive to oxygen.
- It develops in anaerobic conditions at 37-43°C, pH 7.4-7.6 in casein, carbohydrate, meat-peptone environments.
- On blood-sugar agar, it forms semi-transparent colonies with granular and fringed edges.
- In the depth of the sugar agar column, it forms colonies with a compact center similar to cotton balls or snowflakes.
- Most strains produce hemolysis on blood agar.
- When it develops in liquid nutrient media (Kitt-Tarotsi, Wilson-Blair), turbidity is observed along with the formation of gas, and then sedimentation.



***Clostridium novyi***  
Blood agar



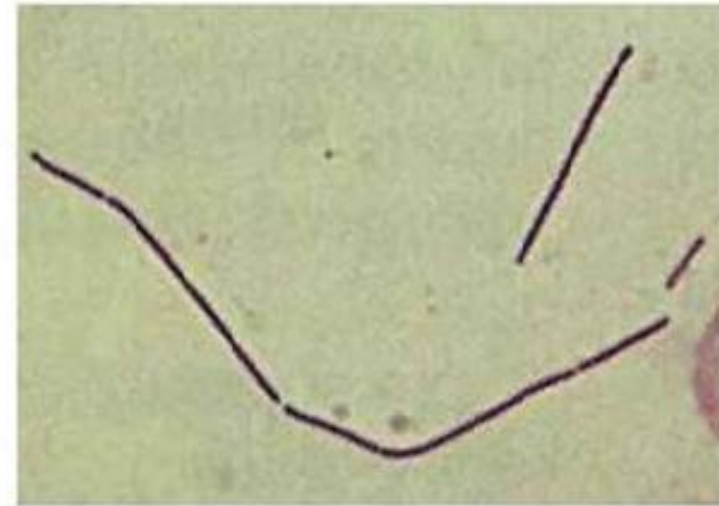
## *Clostridium novyi* *antigen structure and virulence factors:*

- According to the antigenic properties of the exotoxins it synthesizes, it is divided into serotypes A, B, C and D.
- C.novyi secretes a toxin with a complex composition and broad biological activity. The toxin consists of 8 factors with lethal, necrotic and hemolytic effects. They are denoted by Greek letters alpha ( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ), delta ( $\delta$ ), epsilon ( $\epsilon$ ), and mu ( $\mu$ ).
- Toxins lead to the formation of jelly-like edema by increasing the permeability of blood vessels.

# *Clostridium septicum*

## *morpho-biological characteristics:*

- It is a Gram positive 3-4x1.0-1.5  $\mu\text{m}$  polymorphic rod-shaped bacterium. Sometimes the forms of filamentosus up to 50  $\mu\text{m}$  in length are found.
- Unlike *C. perfringens*, it is motile and does not form a capsule.
- Spores are subterminal, sometimes central.



*C. septicum*

# *Clostridium septicum*

## *cultural characteristics:*

- It is obligate anaerobic.
- It grows under anaerobic conditions at 37-43°C, pH 7.4-7.6 in casein and meat-peptone media supplemented with 0.5% glucose.
- On glucose-blood agar, it forms colonies surrounded by a delicate zone of hemolysis, reminiscent of "woven threads".
- In the depth of the sugar agar column, they form colonies with a compact center with a fringe around the edges.
- When it develops in liquid nutrient media (Kitt-Tarozzi, Wilson-Blair), turbidity is observed along with the formation of gas, and then sedimentation.



***Clostridium septicum***  
**glucose-blood agar**

# *Clostridium septicum*

## virulence factors:

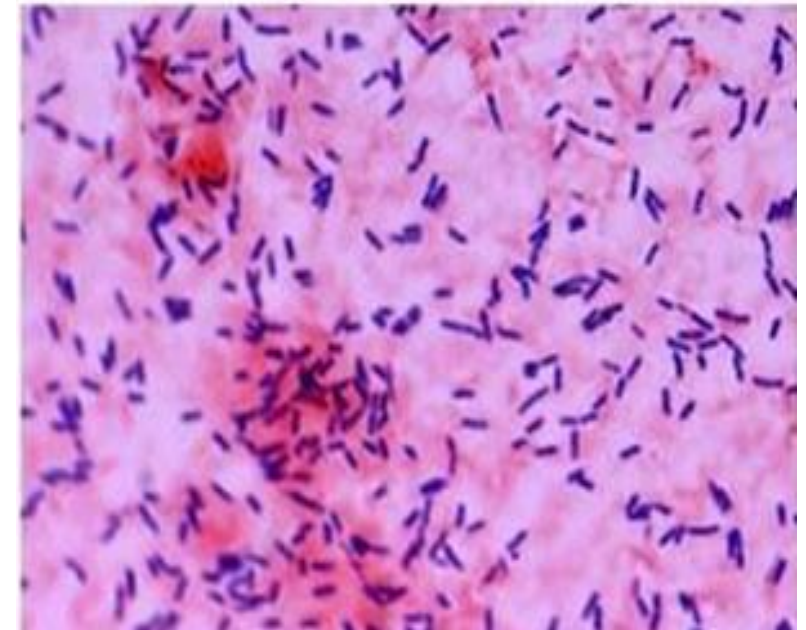
- *C.septicum* secretes 4 exotoxins with lethal, necrotic and hemolytic effects, denoted by Greek letters:
- $\alpha$  -toxin (lecithinase)
- $\beta$ -toxin (DNA-ase)
- $\gamma$ -toxin (hyaluronidase)
- $\delta$ -toxin (oxygen-sensitive hemolysin)



## *Clostridium histolyticum* - "tissue-dissolving bacillus"

### *morpho-biological characteristics:*

- It is a Gram-positive rod-shaped bacterium measuring  $2-3 \times 0.5-1.0 \mu\text{m}$ . They are found in pairs or in chains.
- Unlike *C.perfringens*, it is motile and does not form a capsule.
- Spores are located in the subterminal position.



*Clostridium histolyticum*

# *Clostridium histolyticum*

## *cultural characteristics:*

- It is an aerotolerant anaerobe.
- It develops in anaerobic conditions in casein and meat-peptone environments.
- On blood agar, it forms transparent, convex, shiny colonies with a diameter of 0.5-1 mm, surrounded by a delicate zone of hemolysis.
- In the depth of the sugar agar column, it forms pea-like or fringed colonies with a compact center.
- As a result of proteolysis of pieces of meat and liver in the Kitt-Tarozzi medium, it causes complete turbidity of the medium.



***Clostridium histolyticum***  
Blood agar

# *Clostridium histolyticum*

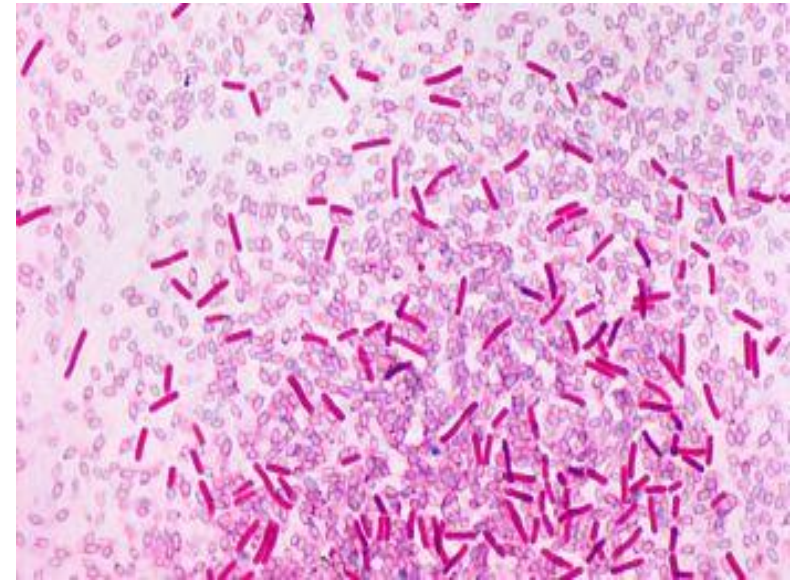
## virulence factors:

- *C.histolyticum* synthesizes a lethal, necrotic and hemolytic toxin with high biological activity:
- $\alpha$  -toxin (lecithinase)
- $\beta$ -toxin (collagenase)
- $\gamma$ -toxin (proteinase)
- $\delta$ -toxin (elastase)
- $\varepsilon$ -toxin (oxygen-sensitive hemolysin, similar to streptococcal O-streptolysin)

# *Clostridium sordellii*

## *morpho-biological and cultural characteristics: :*

- It is a Gram positive 2-4x0.6-1.0  $\mu\text{m}$ , motile rod-shaped bacterium.
- It is a facultative anaerob.
- After 1-2 days of incubation in blood-sugar agar, they form convex, grayish colonies with rough edges.
- Causes hemolysis in blood agar.
- Development in liquid nutrient media (Kitt-Tarozzi, Wilson-Blair) is accompanied by the formation of mucus.



***Clostridium sordellii***



## LABORATORY DIAGNOSIS

**A. Hematological investigation:** Not significant

**B. Bacteriological Investigation:**

**Specimen:** Wound swabs, necrosed tissue, muscle fragments, exudates from active parts etc.

**1. Microscopy:** Gram +ve, non-motile, capsulated bacilli.

-Spores are rarely observed in *Cl.perfringens*

**2. Culture:**

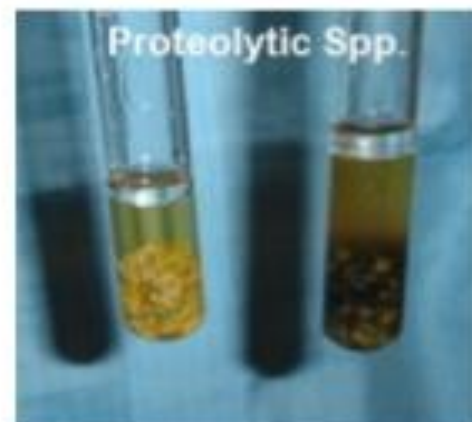
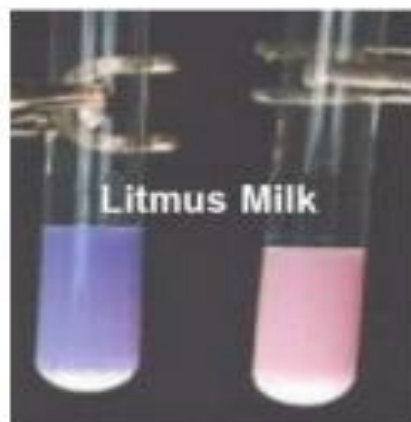
➤ **On RCM** → meat turned pink but not digested

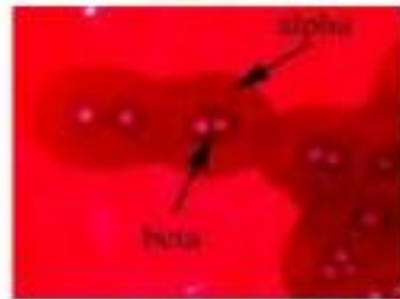
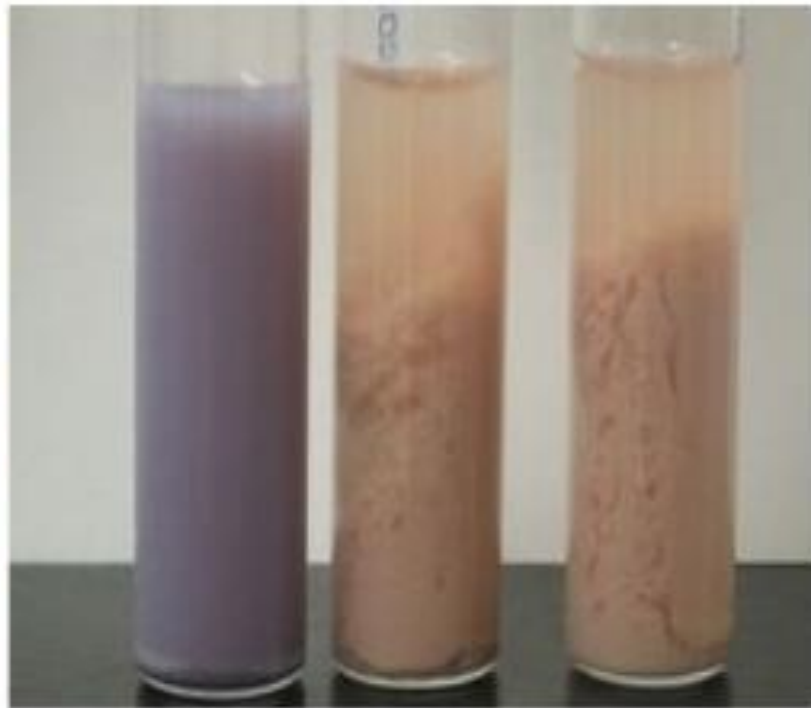
➤ **On blood agar** → target hemolysis

**3. Biochemical reactions:** As discussed above

## CULTURAL CHARACTERISTICS

- Clostridia are anaerobic.
- Optimum temp. for growth is 37°C; pH 7-7.4.
- Robertson's cooked meat broth is useful medium.
- Most species produce gas in this medium
- Saccharolytic species turn meat pink.
- Proteolytic species turn meat black with foul smell.
- Robertson's cooked meat broth is ideal; meat is turned pink but not digested with sour odor.
- Stormy fermentation of lactose in litmus milk; the acid coagulates casein-acid clot.
- On BAM: Target haemolysis





*Left to right:*

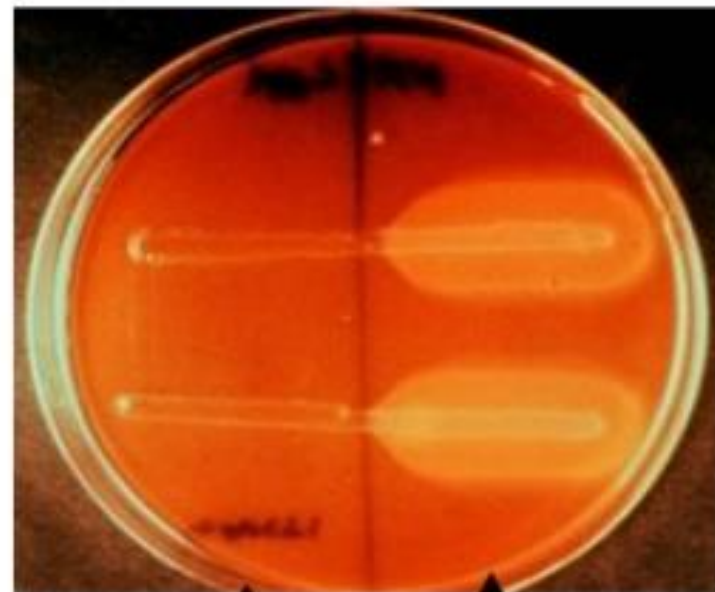
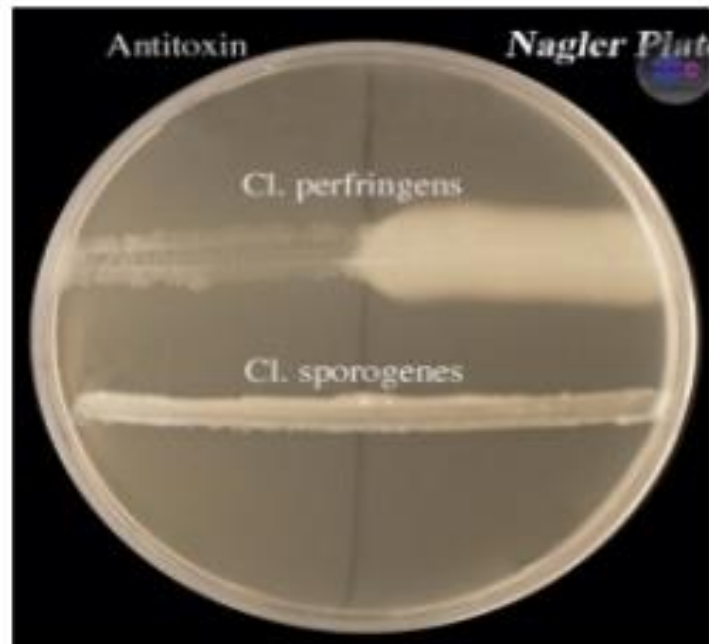
**a.RCM:** Meat turned pink but not digested

**b.Litmus Milk:** Stormy fermentation & acid clot in Litmus milk

**c.BAM:** Target hemolysis

## 4. Nagler's Reaction

- Rapid detection of *Cl. perfringens* from clinical sample
- Done to detect the lecithinase activity of alpha toxin
- Characteristic opalescence is produced around colonies in +ve test due to breakdown of lipoprotein complex in the medium



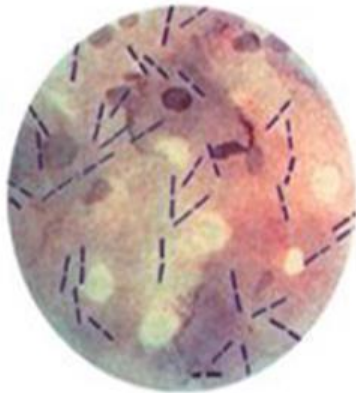
+ Antitoxin      No antitoxin



Species	Egg – yolk agar		Hydrolysis of gelatin	Digestion of casein	Indole production	Acid production			
	Lecithinase	Lipase				Glucose	Lactose	Sucrose	Maltose
<i>C. tetani</i>	-	-	+	-	✓	-	-	-	-
<i>C. botulinum</i> I	-	+	+	+	-	+	-	-	+
<i>C. chauvoei</i>	-	-	+	-	-	+	+	+	+
<i>C. septicum</i>	-	-	+	+	-	+	+	-	+
<i>C. novyi</i> A	+	+	+	-	-	+	-	-	+
<i>C. perfringens</i>	+	-	+	+	-	+	+	+	+

## PROPHYLAXIS & TREATMENT

- 1.Surgery:** All damaged tissue should be removed, wounds irrigated to remove clots, necrotic tissue & foreign materials, **excision** of affected parts in EGG.
- 2.Antibiotics: Metronidazole** given **intravenously** before surgery & repeated **8 hourly for 24 hrs.**  
-Broad spectrum antibiotics in combinations (like metronidazole+gentamycin+amoxicillin)are effective.
- 3.Antitoxins:** Passive immunization with **AGS**  
**3 doses-**     **1 intravenous** dose followed by  
                         **2 intramuscular** doses at **6hrs.** interval



Gram-positive  
'boxcar shaped' bacilli

Uninoculated tube



Inoculated with  
*C. perfringens*

Litmus milk test with 'stormy clot reaction'



Target hemolysis  
(double zone of hemolysis)

# *Clostridium Perfringens*

Anti-alpha toxin

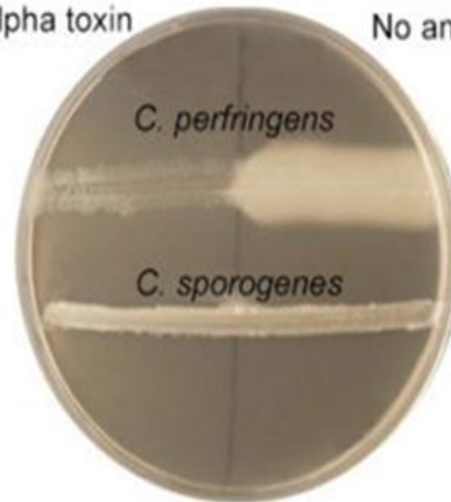
No anti-toxin

*Streptococcus agalactiae*



*Clostridium perfringens*

Reverse CAMP test



Nagler Test



Gas gangrene

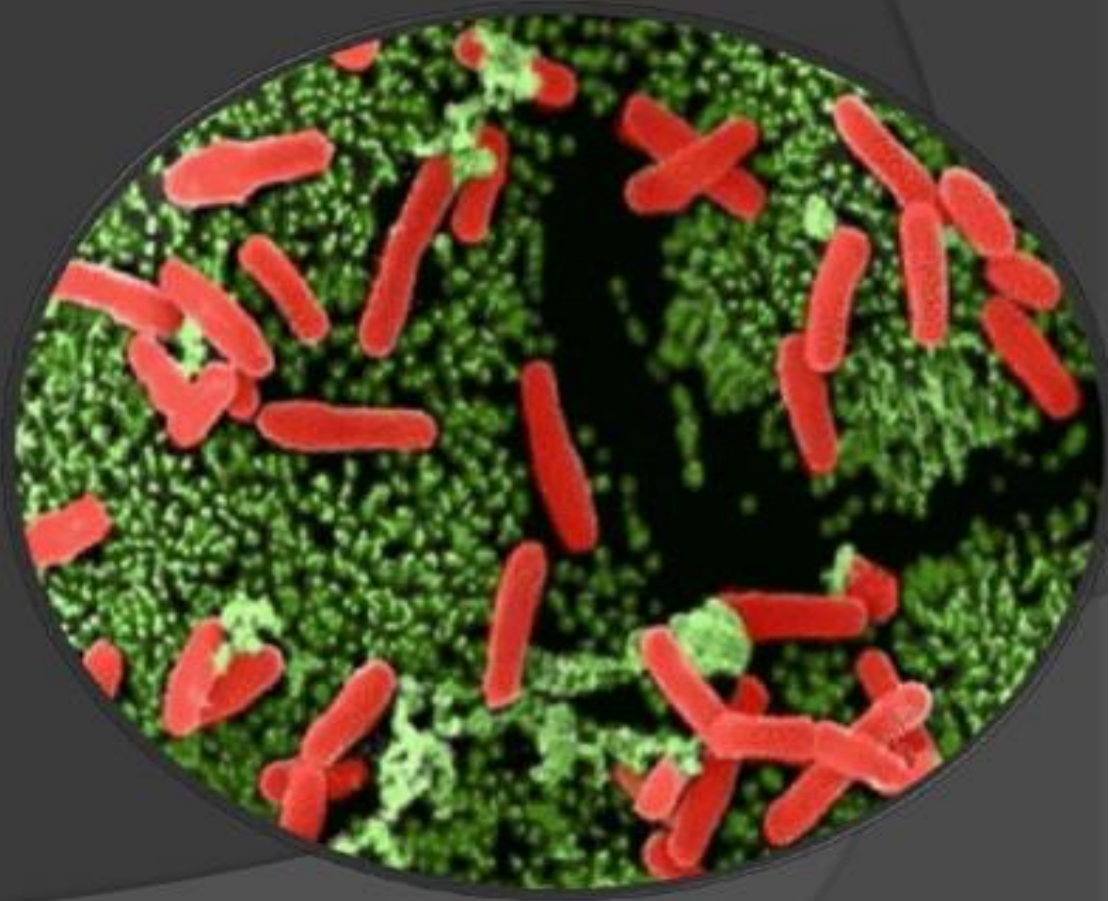
## ***C. difficile***

- Gram + anaerobic rod.
- Found in normal flora
- Ultimate opportunistic pathogen
- Difficult to determine cause as the organism is ubiquitous
- Not difficult to culture



# *Clostridium difficile*

- ***Clostridium difficile***  
(Greek *kloster* (κλωστήρ), spindle, and Latin *difficile* difficult), also known as "CDF/cdf", or "C. diff", is a species of Gram-positive bacteria of the genus *Clostridium* that causes diarrhea and other intestinal disease when competing bacteria are wiped out by antibiotics.



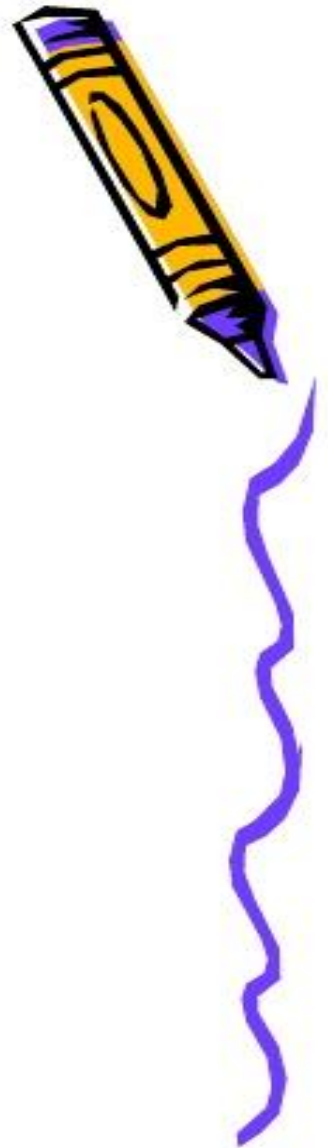
# Several Antibiotics cause pseudomembraneous colitis

- ◉ Nearly all antibiotics can cause antibiotic-associated diarrhea, colitis or **pseudomembraneous colitis**. The antibiotics most commonly linked to antibiotic-associated diarrhea :



## Other risk factors

- advanced age,
- hospitalization,
- inflammatory bowel disease,
- chemotherapy, and
- immunosuppression.





# Pathophysiology

- disruption of the normal bacterial flora of the colon
- colonization with *C. difficile*
- release of toxins
- mucosal damage and inflammation.



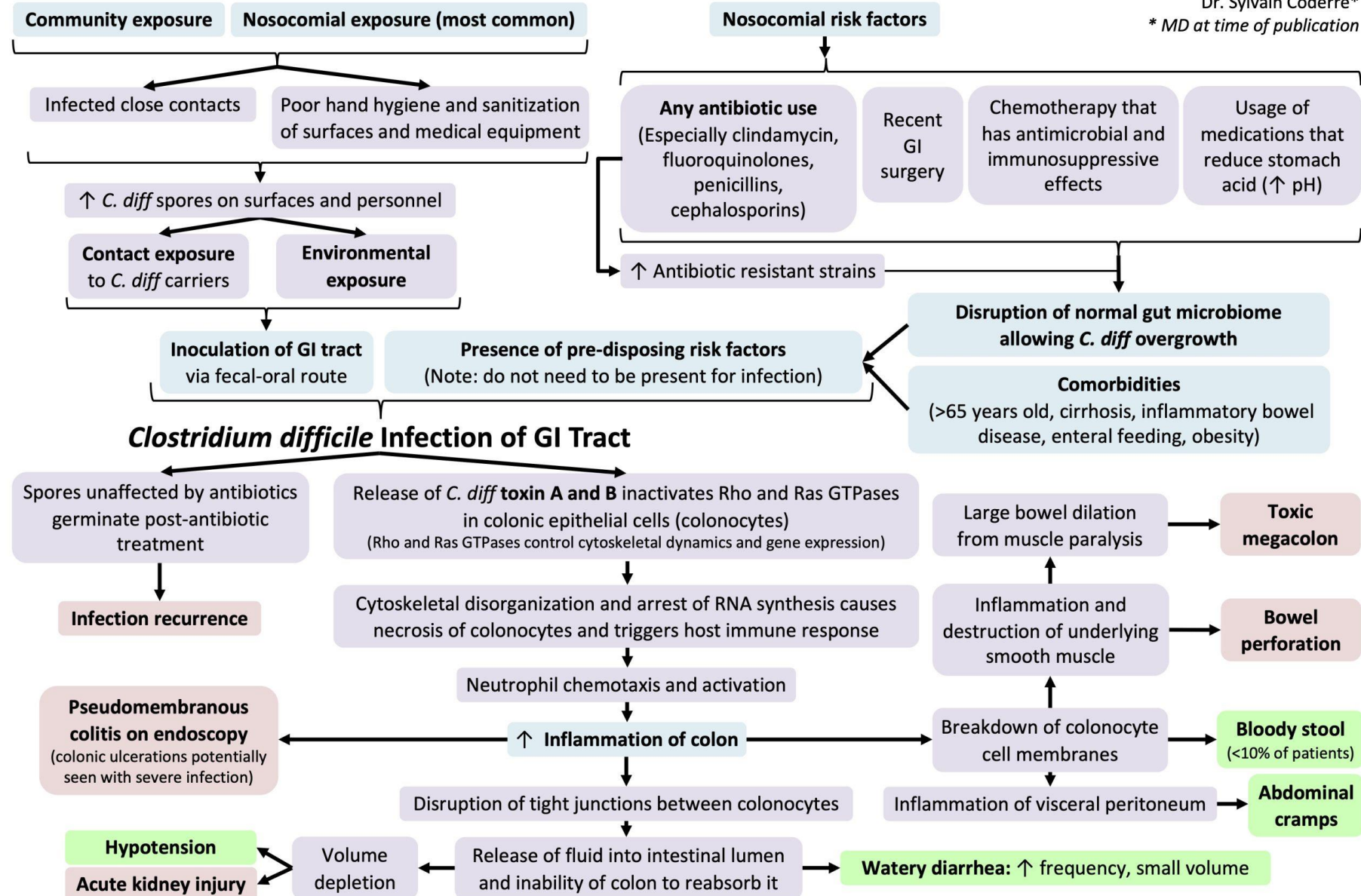


## ***C. difficile Virulence Factors***

<b>Virulence Factor</b>	<b>Biologic Activity</b>
Enterotoxin (toxin A)	Produces chemotaxis; induces cytokine production with hypersecretion of fluid; produces hemorrhagic necrosis
Cytotoxin (toxin B)	Induces depolymerization of actin with loss of cellular cytoskeleton
Adhesin factor	Mediates binding to human colonic cells
Hyaluronidase	Produces hydrolytic activity
Spore formation	Permits organism's survival for months in hospital environment

# Clostridium difficile (C. diff) Infection

**Authors:** Ryan Brenneis, Sravya Kakumanu  
**Reviewers:** Yoyo Chan, Sean Doherty, Vina Fan, Ben Campbell, Dr. Steve Vaughan\*, Dr. Sylvain Coderre\*  
 \* MD at time of publication



## ***C. difficile* Diagnosis**

- ✓ Cytotoxin - stool
- ✓ Culture
- ✓ *C. difficile* antigen – latex agglutination

## C. difficile

- Culture: standard test for Clostridia include: indole, sugars, lecithinase, catalase (usually neg.)
- *C. difficile*: Latex agglutination
- ✓ Stool buffered and centrifuged
- ✓ Drop on slide of stool supernatant
- ✓ add 1 drop latex detection reagent. Latex particles coated with rabbit antibody to *C. difficile* antigen.
- ✓ In presence of *C. difficile* clumps can be seen by eye.



## Culture

### Inoculate

anaerobe blood agar -- 2-3 days

egg yolk medium -- 2-3 days

Incubation temp. = 30 C except *C. perfringens*

Inoculate cooked meat medium - (broth with meat particles)

## ***C. difficile: Culture***

- ✓ Heat to destroy vegetative cells
- ✓ Alcohol spore selection for heat labile spores.
- ✓ **Clinical syndromes: most serious is Pseudomembranous colitis (PMC)**
- ✓ Pseudomembranous colitis brought about by destruction of the other indigenous intestinal flora.
- ✓ Ranges from mild to serious.
- ✓ PMC is self-limiting.

## Treatment

- Stop antibiotic causing disease
- ✓ Metronidazole, Vancomycin
- Relapses due to resistant spores.
- ✓ retreatment with same antibiotic
- neutralization with specific antitoxin obtained commercially
- ✓ amount of toxin present can be determined by a dilution series of the stool sample.

## ANAEROBIC GRAM NEGATIVE BACILLI

- Bacteroides ( most common )
- Prevotella
- Porphyromonas
- Fusobacterium
- Bilophila

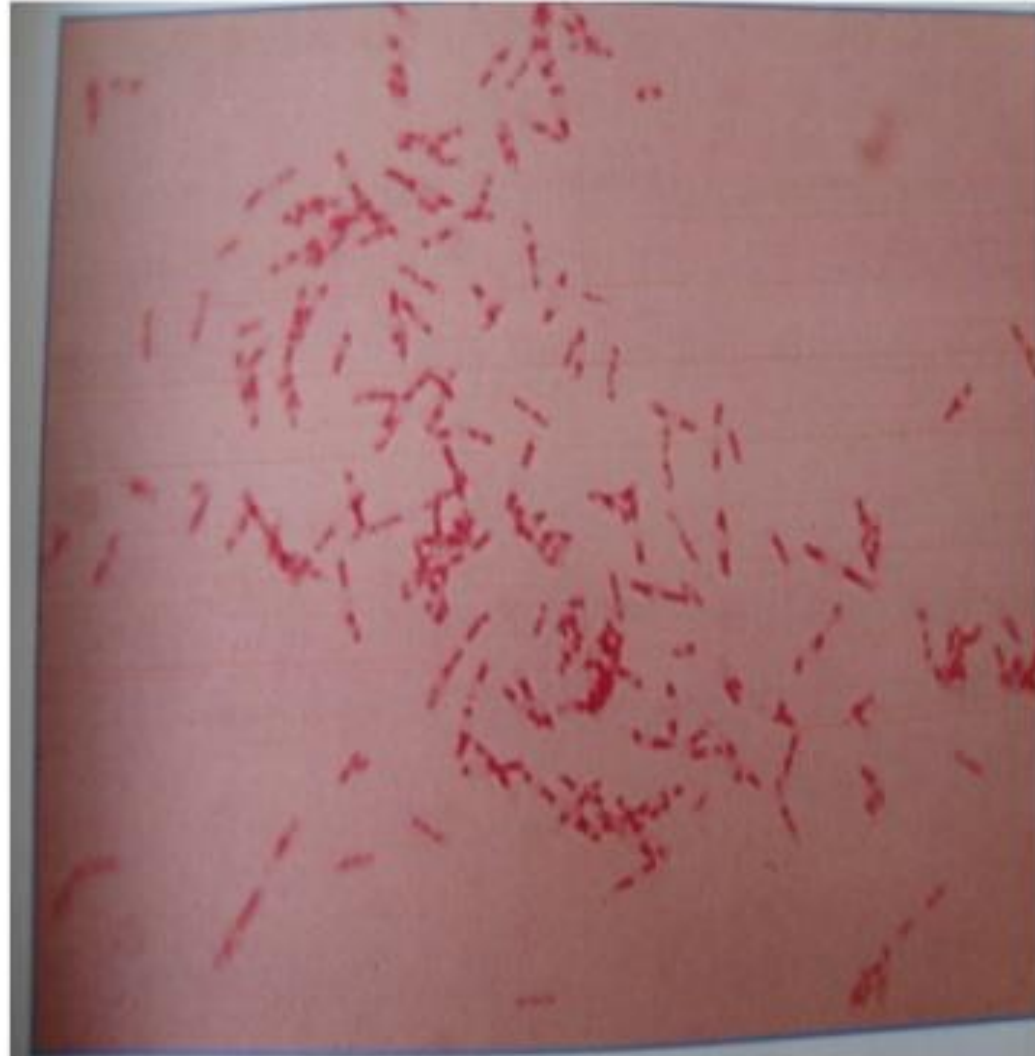
All non motile



# 1. Bacteroides group

- B. fragilis (most common)
- B.thetaiotaomicron

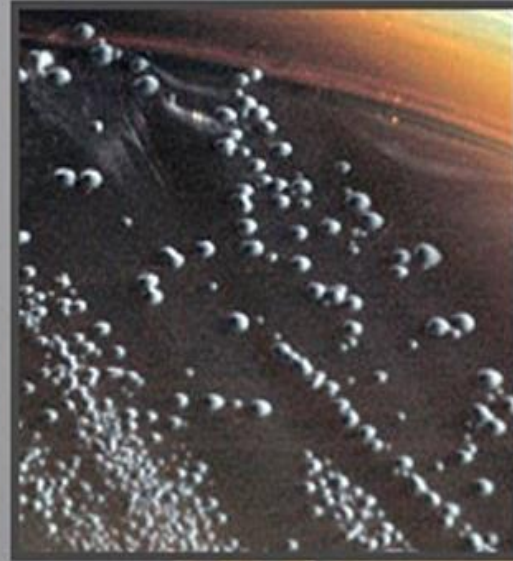
- Gram negative bacilli with **rounded ends**
- 1.5-9  $\mu\text{m}$  vs 0.5-0.8  $\mu\text{m}$
- Nonmotile
- Broth culture :  
    pleomorphic with  
    vacuoles
- Capsules



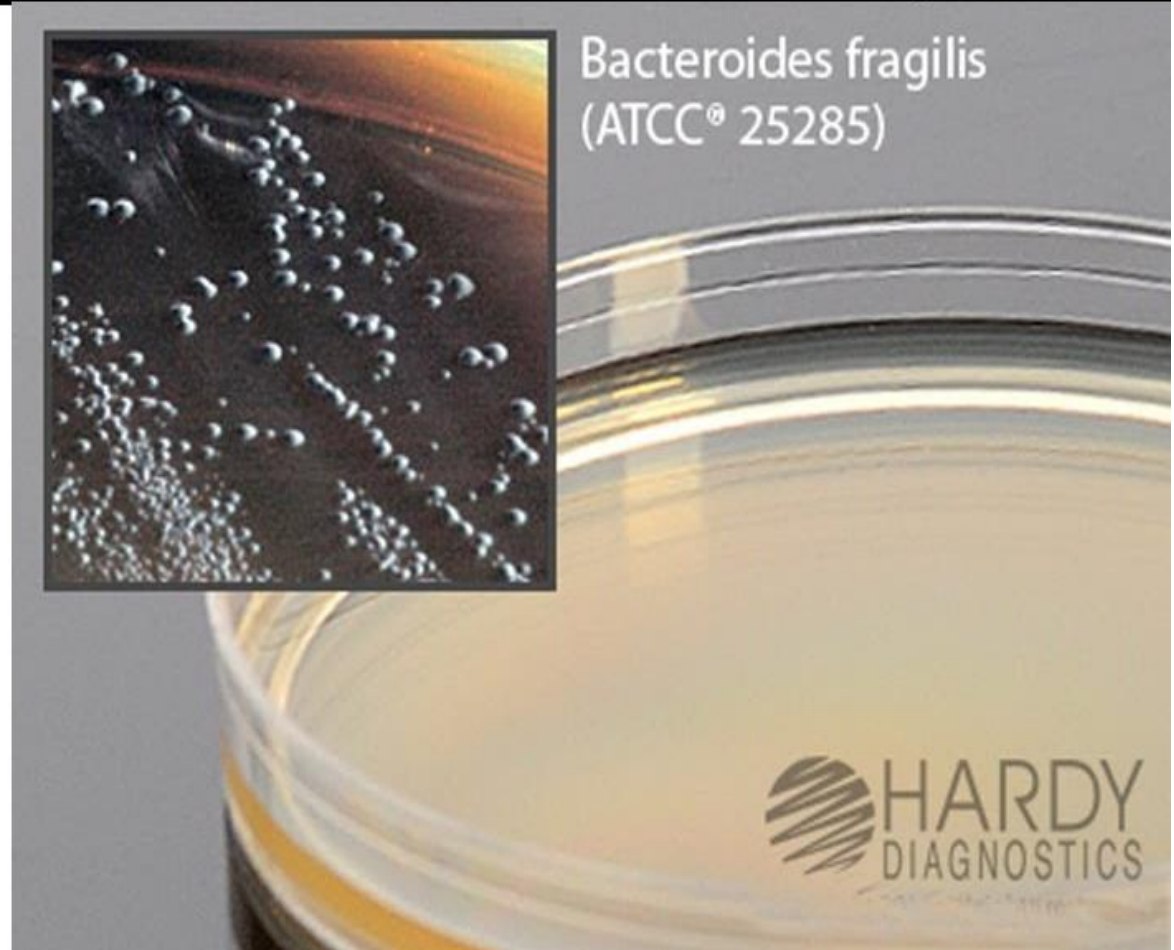
# Bacteroides Bile Esculin Agar



*Bacteroides fragilis*



*Bacteroides fragilis*  
(ATCC® 25285)



## BACTEROIDES FRAGILIS GROUP

- Non-motile
- On aerobic blood agar, colonies are non-hemolytic and gray with an entire margin and ring-like structures
- Saccharolytic
- Extremely virulent and can cause widespread tissue destruction
- Has a capsule and also produces several enzymes
- No pigment or spore formation