

Spore forming Gram Positive Bacilli Anaerobic Bacteria.....(*Clostridium* spp.)

Objective:

Upon completion of this lecture, you will be able to:

- 1- Determine the characteristic features of *Clostridium* spp.
- 2- Recognize the pathogenic species.
- 3- Distinguish the virulence mechanism to cause infections.
- 4- Differentiate between related clostridial species.
- 5- Describe the methods for lab. Diagnosis.

General Characteristic Feature

- Large Gram-positive rods.
- Obligate anaerobes.
- The spores are usually wider than the rods, and are located centrally, terminally or subterminally.
- Most clostridia are motile by peritrichous flagella.
- Clostridia are naturally inhabited the soil and the intestinal tracts of humans and animals.

Clostridium species

1. *Clos. botulinum*..... Botulism
2. *Clos. tetani*..... Tetanus
3. *Clos. perfringens*.... Gas gangrene; Food poisoning.
4. *Clos. difficile*:..... Pseudomembranous colitis
5. *Clos. septicum* , *Clos. novy* , *Clos. sporogenes*

Cultural Conditions

- The clostridia grow well on the blood-enriched media.
- Anaerobic bacteria will not grow in the presence of oxygen and are killed by oxygen or toxic oxygen radicals.
- Low oxidation-reduction potential (Eh) favor growth of anaerobes.

- **Colony Forms:**

1. Some produce large raised colonies (eg, *C.perfringens*).
2. Others produce smaller colonies (eg, *C.tetani*).
3. Some form colonies that spread on the agar surface.
4. Many clostridia produce a zone of hemolysis on blood agar.
5. *C.perfringens* typically produces **multiple zones of hemolysis** around colonies.

- **Growth Characteristics:**

1. Ferment a variety of sugars and digest proteins.
2. Milk is turned acid, digested and undergoes a "stormy fermentation" (eg, *C perfringens*).
3. Various enzymes are produced.

***Clostridium botulinum* (Botulism)**

- Worldwide in distribution, found in soil.
- Foodborne botulism is an intoxication, that is, the toxin is ingested with food.
- *C. botulinum* toxins are among the most toxic substances known.
- Types of *C. botulinum* are distinguished by the antigenic type of toxin they produce.
- Neurotoxin: Pharmaceutical for human use (agent acting on the nervous system)
- Bacterium: biological warfare agent
- Botulin toxin A is used in the treatment of spastic muscular conditions.
- It is also used for cosmetic purposes to reduce wrinkles.

Determinants of Pathogenicity

- **Botulinum Toxin**

- 1- It is generically designated as **botulin**.
- 2- The toxin is a 150,000-MW protein that is cleaved into 100,000-MW and 50,000-MW proteins linked by a disulfide bond.
- 3- The various strains actually produce eight toxins (**A, B, C1, C2,... G**).
- 4- Types **A, B, and E** cause poisoning in humans.

- 5- Toxins A through F are **neurotoxins** that interfere with neurotransmission at the peripheral cholinergic synapses by preventing the release of acetylcholine causing **flaccid paralysis**.
- 6- Destroyed by heating for 20 min. at 100°C.
- 7- The lethal dose for a human is probably about 1–2µg.

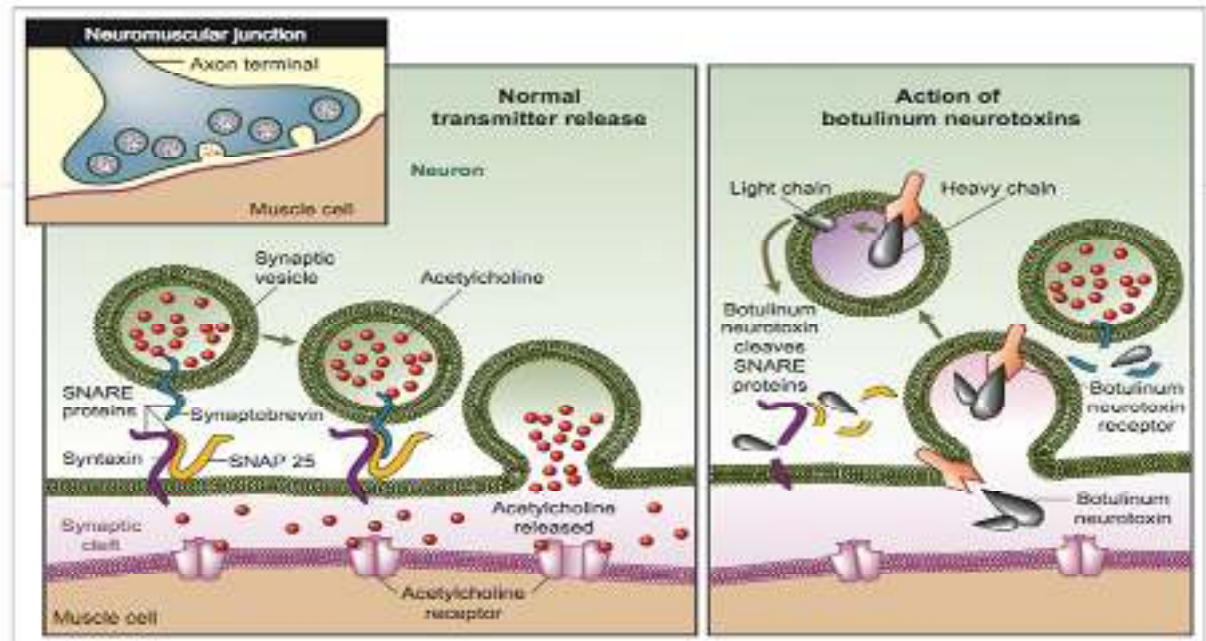


Figure-1- Action of Botulinum toxin

Pathogenesis

- It is an intoxication.
- Ingestion of food (spiced, smoked, vacuum-packed, or canned alkaline foods) in which spores of *Clos. botulinum* germinate; under anaerobic conditions, vegetative forms grow and produce toxin.
- The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions (see above). **Flaccid paralysis** results.

Clinical Diseases

1- Foodborne botulism

- Incubation period: 18-24 hrs.
- Mortality is high.

2- Wound botulism

- Develops from contaminated wounds.

3- Infant botulism

- Occurs in the first months of life.
- Weakness, signs of paralysis, ("**floppy baby**").
- *C. botulinum* and its toxin are found in feces. May be caused by ingestion of the bacteria or spores which grow in the gut and produce toxin.
- Feeding of honey has been implicated as a possible cause. So honey should not be given to children during the first year of life

Diagnostic Laboratory Tests

- 1- **Toxin detection** (in feces or serum from the patient and in leftover food) by means of:

A- Mice injected intraperitoneally.

B- Neutralization test: The antigenic type of toxin is identified by neutralization with specific antitoxin in mice.

- 2- **Cultivation** of *C. botulinum* from patient feces and implicated food.

Treatment

- Urgent administration of a **polyvalent antitoxin**.?....Why?
- Adequate ventilation.

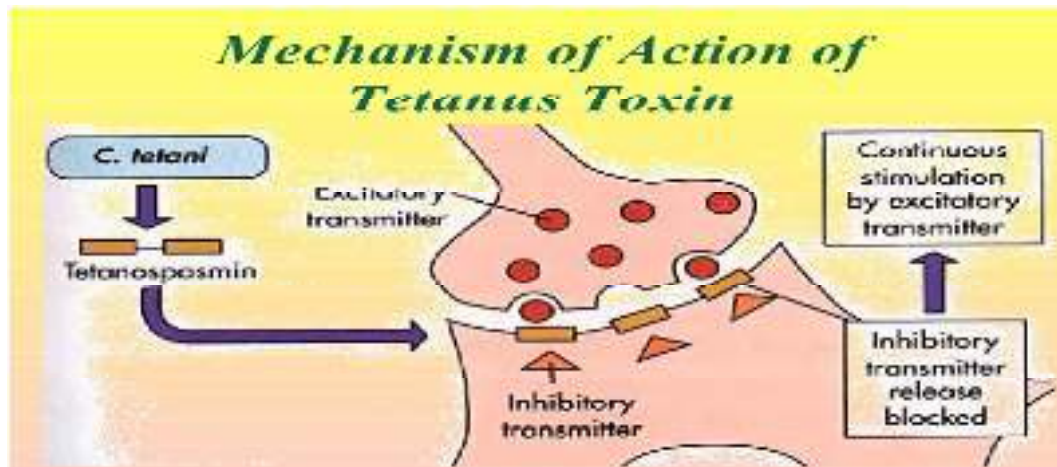
Clostridium tetani (Tetanus)

- Worldwide distributed in the soil, feces of horses and other animals.
- Produce a terminal spore, (drumstick appearance).
- Tetanus (**lockjaw**) is an acute clostridial disease.
- All produce the same antigenic type of neurotoxin, **tetanospasmin**.
- The portal of entry is a wound site.

Determinants of Pathogenicity

Toxin

- *Clos. tetani* produce a potent neurotoxin; **tetanospasmin**.
- Tetanospasmin (MW 150,000) is cleaved into two peptides (MW 50,000 and 100,000) linked by a disulfide bond.
- Toxin is under the control of a plasmid gene.
- The toxin initially binds to receptors on the presynaptic membranes of motor neurons, and then migrates to the spinal cord and brain stem.
- The toxin diffuses to terminals of inhibitory cells.
- Release of the inhibitory glycine and aminobutyric acid is **blocked**.
- Muscle spasms, and **spastic paralysis** result.
- Extremely small amounts of toxin can be lethal for humans.



Pathogenesis

- *Clos. tetani* is **not an invasive** organism.
- The disease is a **toxemia**.
- Contamination of devitalized tissue (wound, umbilical stump, surgical suture) with the spores.
- Germination of the spores.
- Release of tetanospasmin.
- The toxin reaches CNS fixed to gangliosides in spinal cord and exerts its actions.

- Germination of the spore and development of vegetative organisms that produce toxin are aided by:
 - 1- Necrotic tissue
 - 2- Pyogenic infections
 - 3- low oxidation-reduction potential.

Types of tetanus:

- 1- Generalized tetanus Neonatal tetanus
- 2- Localized tetanus Cephalic tetanus.

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

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.
- It's often called the silent killer, since infants often die before their birth is recorded.

Lab. Diagnosis

- Clinical picture and a history of injury.
- Anaerobic culture of tissues from contaminated wounds.
- Proof the isolation of *Cl. tetani* by toxigenicity test.

Treatment

Tetanus is a totally preventable disease.

- **(Prevention is much more important than treatment)**
 - 1- Proper care of wounds.
 - 2- Prophylactic use of **antitoxin**.
The **toxin** is neutralized with shots of tetanus immunoglobulin, TIg.

3- Administration of **penicillin**.

4- Active immunization with **toxoid** (toxin detoxified with formalin).

Tetanus toxoid-In young children- is part of routine childhood DTaP (diphtheria, tetanus and pertussis) immunizations.

Questions

- 1- List the species of *Clostridium*?
- 2- Give two diagnostic feature of *Clos. tetani*?
- 3- Differentiate between *Cl. tetani* and *Cl. botulinum*?
- 4- The following are true about *Clostridium tetani*:
 - a. it is a Gram positive bacilli with terminal spore
 - b. tetanus can occur weeks after the initial infection
 - c. the symptoms of tetanus is produced by an endotoxin
 - d. tetanus antitoxin is ineffective when the toxin enters the nervous tissue
 - e. gas gangrene can result from *Clostridium tetani* infection

Note: All figures and pictures are available freely in web during preparation of this lecture.