

Medical Bacteriology
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Spore-Forming Gram-Positive Bacilli: Bacillus and Clostridium Species

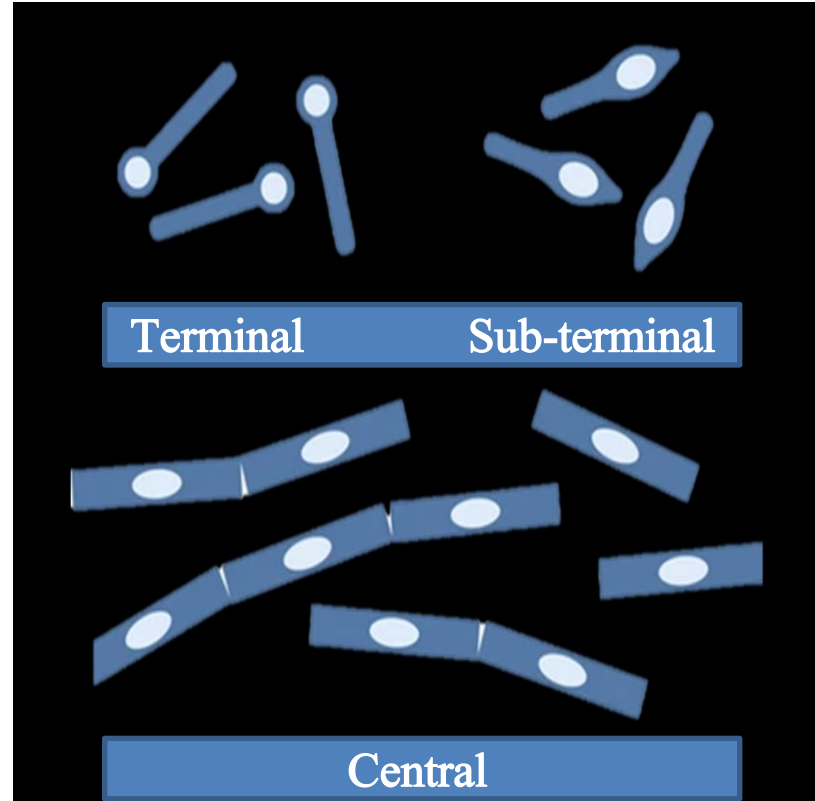
- These bacilli are ubiquitous, because they form **spores (can survive in the environment for many years)**.

Spore forming bacilli belong to two genera:

- **Bacillus:** They are obligate aerobes
- **Clostridium:** They are obligate anaerobes.

Spore

- ✓ In clostridia, the spores are wider than the vegetative bacteria giving rise to swollen or spindle-shaped appearance.
- ✓ Spore formation occurs in unfavorable conditions.
- ✓ In the various species, the spore is placed centrally, subterminally, or terminally.



Bacillus

- The genus *Bacillus* includes large **aerobic**, gram-positive rods occurring in chains.
- Most members of this genus are **saprophytic organisms** prevalent in soil, water and air.
- *B. cereus* can grow in foods and cause food poisoning by producing either an enterotoxin (diarrhea) or an emetic toxin (vomiting).
- *B. cereus* may caused disease in immunocompromised humans.
- *B. anthracis* is causes anthrax.

Bacillus cereus

- It is a normal habitant of soil, also widely isolated from food items, such as vegetables, milk, cereals, meat .
- **Manifestations:**
 - ✓ **Food poisoning:** It produces two types of toxins; diarrheal toxin and emetic toxin
 - ✓ **Ocular disease:** Causes severe keratitis following trauma to the eye.
 - ✓ **Other conditions:** It rarely causes systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia in immunocompromised humans
- **Laboratory diagnosis:** motile bacilli, spores are located in the center, noncapsulated
 - ✓ It can be isolated from patient stool by using selective media such as; MYPA (Mannitol, egg yolk, phenol red, polymyxin and agar)

<i>B. cereus</i>	Diarrheal type	Emetic type
Incubation period	8–16 hours	1–5 hours
Toxin	Secreted in intestine (Similar to <i>Clostridium perfringens</i> enterotoxin)	Preformed toxin (similar to <i>S.aureus</i> enterotoxin)
	Heat labile	Heat stable
Food items contaminated	Meat, vegetables, dried beans, cereals	Rice (fried rice)
Clinical feature	Diarrhea, fever, rarely nausea	Vomiting, abdominal cramps

Bacillus anthracis

- ✓ *B.anthraxis* is the causative agent of an important zoonotic disease called **anthrax**.
- ✓ Could used as biological weapon.
- ✓ It occurs via inhalation of anthrax spores from contaminated products.
- ✓ **Virulence Factors and Pathogenesis:** Pathogenesis of anthrax is due to two important virulence factors; anthrax toxin and capsule.

Virulence Factors

Anthrax capsule

- ✓ *B.anthraxis* has a polypeptide capsule made-up of polyglutamate
- ✓ Capsule is plasmid coded.
- ✓ It inhibits complement mediated phagocytosis.
- ✓ *B.anthraxis* isolates that do not produce a capsule are not virulent and do not induce anthrax.

Anthrax toxin

- ✓ It is a triple toxin, consisting of three fragments: **Edema factor**, **Protective factor** and **Lethal factor**
- ✓ These fragments are not toxic individually, but in combination, they produce local edema and generalized shock.
- ✓ Toxin synthesis is controlled by a plasmid.
- ✓ Loss of plasmid makes the strain avirulent.

Pathogenesis and Clinical Manifestations

- ✓ Anthrax is primarily a zoonosis.
- ✓ **Human Transmission:** Human beings acquire infection by:
 - **Cutaneous mode:** By spores entering through the abraded skin; seen in people with occupational exposure to animals (**Cutaneous Anthrax**)
 - By inhalation of spores (**Pulmonary Anthrax**)
 - Ingestion of carcasses of animals dying of anthrax containing spores (manifested as bloody diarrhea)(**Intestinal anthrax**)



1. **Direct smear microscopy:** chain of bacilli arranged in **bamboo stick** appearance
2. **Spores** can be confirmed by phase contrast microscope or use of special stains such as hot malachite green.

3. Culture

- ✓ Nutrient agar- Medusa head appearance colony
- ✓ Blood agar- nonhemolytic colonies
- ✓ Selective media- PLET media

4. Biochemical identification

- ✓ Motility test: Nonmotile
- ✓ Gelatin liquefaction- Appear as **inverted fir tree** appearance

5. **Direct fluorescent antibody test:** Detects capsular antigen. It is used for confirmation of diagnosis during bioterrorism outbreaks

6. **Molecular methods:** PCR

Clostridium Species

- ✓ Obligate anaerobic gram-positive bacilli, having bulging spores:
- ✓ Clostridia are saprophytes found in soil, organic matter, and also in intestine of animals including humans
- ✓ Only few infect humans, *such as C. perfringens, C. tetani, C. botulinum and C. difficile*
- ✓ They are motile except *C. perfringens* and *C. tetani*.
- ✓ They are noncapsulated except *C. perfringens* .
- ✓ Most of the clostridia have a sub-terminal spores except: *C. tetani*:
Produces spherical and terminal spore (drum stick appearance)

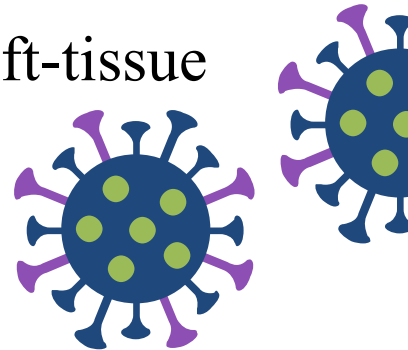
Clostridium Perfringens

- ✓ *C. perfringens* is a saprophyte and commensal in the large intestine of human beings and animals
- ✓ It is capsulated, nonmotile, gram-positive bacillus
- ✓ It bears sub-terminal bulging spores; but the gas gangrene strains do not produce spores.
- ✓ *C. perfringens* is invasive as well as toxigenic.
- ✓ Main Virulence Factors:
 - Alpha toxin is the principle virulence factor for gas gangrene and food poisoning
 - They also produce heat labile enterotoxin

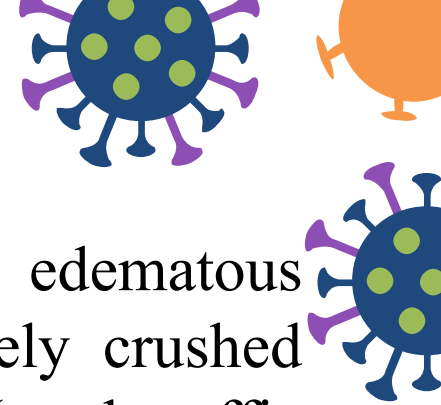
Clinical Manifestations

C. perfringens infections are mostly **polymicrobial** involving other clostridia species. Various manifestations include:

1. **Clostridial Wound Infection:** Simple wound contamination, Anaerobic cellulitis, Anaerobic myositis (gas gangrene)
2. **Clostridial Enteric Infection** Food poisoning, Enteritis necroticans (gas gangrene of the bowel, Necrotizing enterocolitis)
3. **Other Clostridial Infections:** Bacteremia, Skin and soft-tissue infections, Meningitis and brain abscess.



Gas Gangrene



- ✓ Gas gangrene is defined as a rapidly spreading, edematous myonecrosis, occurring in association with severely crushed wounds contaminated with pathogenic clostridia (road traffic accidents, bullet injuries, war injury or invasion of bowel clostridia)
- ✓ The incubation period is variable, depending upon the nature of injury, infective dose and Clostridial species involved
- ✓ like: 10–48 hrs for *C. perfringens*.

Gas Gangrene is Clinically Characterized By

- ✓ Sudden onset of severe pain at the affected site
- ✓ Rapid development of a **foul-smelling** , discharge and Gas bubbles
- ✓ Shock and organ failure develop later.
- ✓ Associated with higher mortality rate (50%)



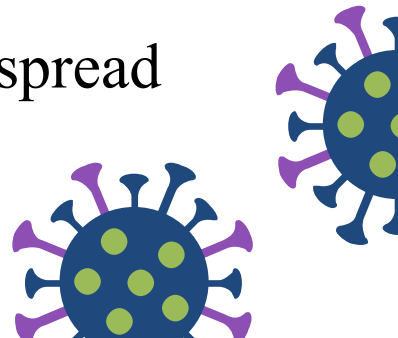
Laboratory Diagnosis

1. **Specimen:** Necrotic tissues, muscle fragments and exudates from deeper wound.
2. **Direct microscopy:** Thick, stubby, boxcar -shaped gram-positive bacilli without spore – suggestive of *C. perfringens*
3. **Culture media within anaerobic conditions:** Robertson cooked meat broth (RCM) and Target hemolysis (double zone hemolysis).



Clostridium tetani

- ✓ *C. tetani* is obligate anaerobic, gram-positive bacillus with terminal round spores (drum stick appearance)
- ✓ It is the causative agent of ‘**tetanus**’ manifested by skeletal muscle spasm and autonomic nervous system disturbance.
- ✓ *C. tetani* is widely distributed in soil, hospital and intestine of man and animals.
- ✓ Tetanus bacilli enter through:
 - Injury like road traffic accidents, unsterile surgery/abortion/delivery, otitis media
 - It is noninfectious: There is no person to person spread
 - Incubation period is about 6–10 days.



C. tetani produces two exotoxins:

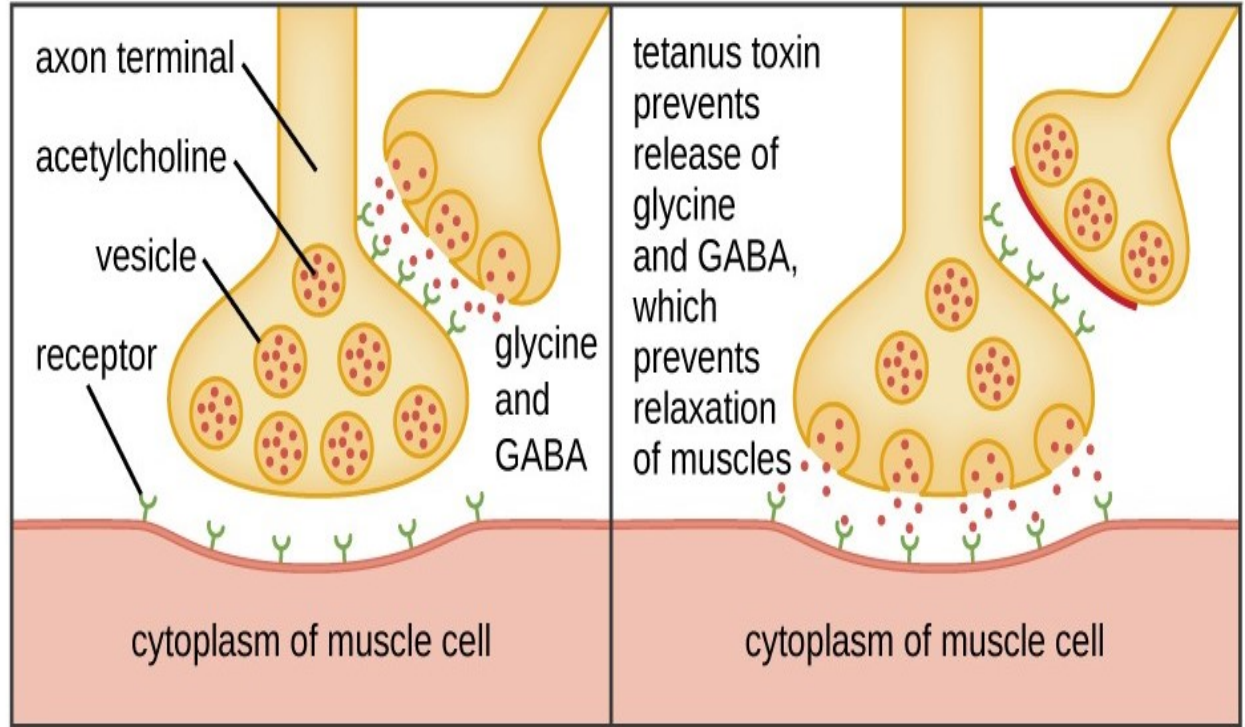
1. **Tetanolysin** is a heat labile, oxygen labile hemolysin. It plays no role in the pathogenesis.
2. **Tetanospasmin** or **tetanus toxin** (TT) is a neurotoxin responsible for the pathogenesis of tetanus: It is oxygen stable but heat labile; coded by plasmid.

Tetanus symptoms:

- ✓ Muscles of the face and jaw are often affected first (due to shorter distances for the toxin to reach the nerve terminals).
- ✓ Tetanus toxin acts at the inhibitory neuron terminals and prevents release of inhibitory neurotransmitter GABA and glycine → leads to spastic contraction



tetanus toxin
(spastic paralysis:
stops uncontrollable
muscle contraction)

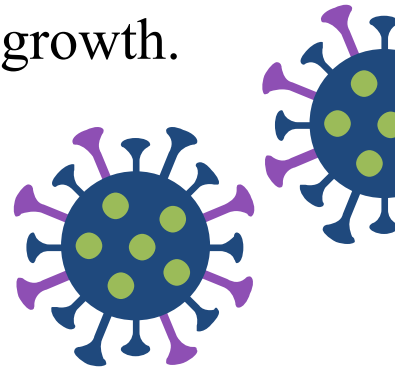


normal mechanism

abnormal mechanism

Laboratory diagnosis

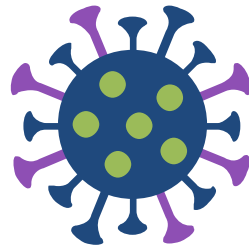
1. **Specimen:** Excised tissue bits from the necrotic depths of wounds.
2. **Gram staining:** gram-positive bacilli with terminal and round spores (drumstick appearance). However microscopy alone is unreliable as it cannot distinguish *C. tetani* from morphologically similar clostridia
3. **Culture:** Culture is more reliable than microscopy:
 - In RCM broth: *C. tetani*, being proteolytic turns the meat black and produces foul odor.
 - Blood agar: *C. tetani* produces characteristic swarming growth.
4. **Toxigenicity Test:** For demonstration of toxin production
5. **In vivo mouse inoculation test:** detects tetanospasmin



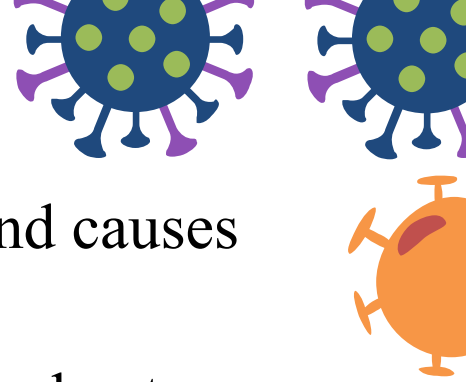
Prevention by Active Immunization (Vaccine)



- ✓ Tetanus toxoid (TT) is used for active immunization.
- ✓ It is available either as **Monovalent vaccine as TT** and **Combined vaccine as DPT**.
- ✓ Primary immunization of children: total seven doses are given at 6, 10 and 14 weeks to 16 yrs.
- ✓ Adult immunization: It is indicated if primary immunization is not administered in childhood. Four doses of TT is given.
- ✓ Site: TT is given deep IM at anterolateral aspect of thigh (children) and in deltoid (adults).
- ✓ Protective titer of tetanus antitoxin is ≥ 0.01 unit/ml.

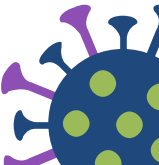
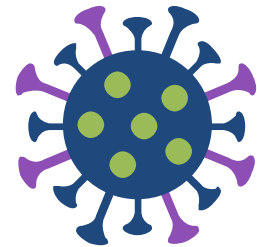
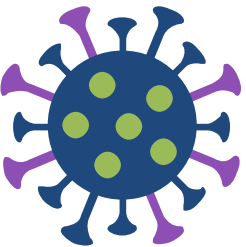


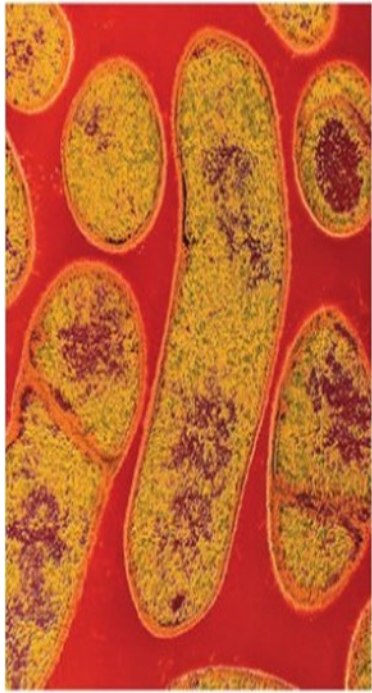
Clostridium botulinum



- ✓ Clostridium botulinum produces botulinum toxin and causes botulism.
- ✓ C. botulinum is noninvasive and the pathogenesis is due to production of powerful neurotoxin ‘botulinum toxin’ (BT)
- ✓ BT probably the most toxic substance known to be lethal to mankind.
- ✓ BT is produced intracellularly, not secreted and appears outside only after autolysis of bacterial cell.
- ✓ BT is synthesized as protoxin, converted into active form by proteolytic enzymes.

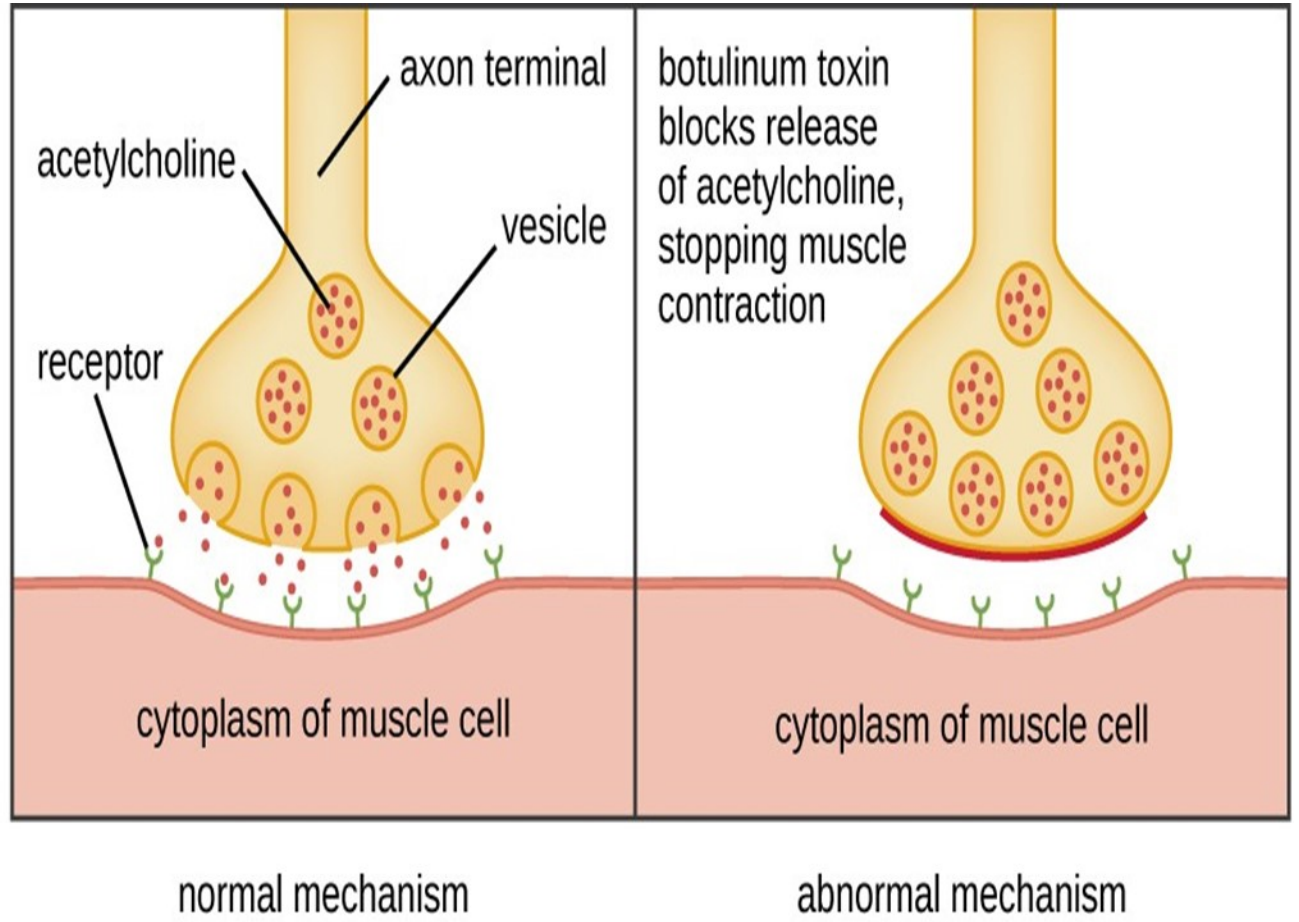
- ✓ Mechanism: BT blocks the release of acetylcholine in neuromuscular junction, which leads to paralysis.
- ✓ Therapeutic uses: As BT produces flaccid paralysis it can be used therapeutically for the treatment of spasmodic conditions.
- ✓ Botulinum toxin targeted SNARE proteins in the neurons inhibits the release of acetylcholine, resulting in lack of muscle contraction and paralysis.



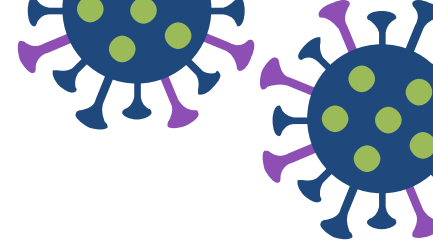


botulinum toxin

(flaccid paralysis: stops muscle contraction)



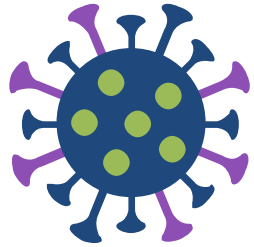
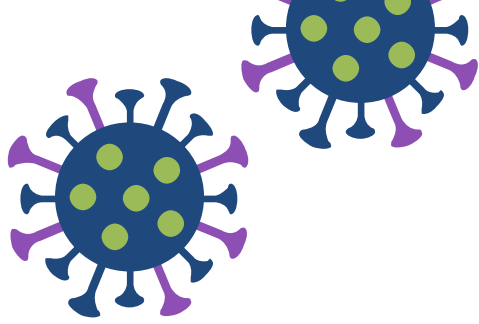
Clinical Manifestations



Types of Botulism

- ✓ **Food borne botulism:** Results from foods contaminated with preformed botulinum toxin; commonly due to consumption of **homemade canned food**.
- ✓ **Wound botulism:** results from contamination of wounds with *C. botulinum* spores.
- ✓ **Infant botulism:** By ingestion of contaminated food (usually honey) with spores of *C. botulinum* in children ≤ 1 year of age. Spores germinate in intestine releasing the toxin. Manifestations include inability to suck and swallow, weakened voice, and floppy neck, extreme weakness (called floppy child syndrome) ; It is usually self-limiting.





Thanks!
Any questions?

